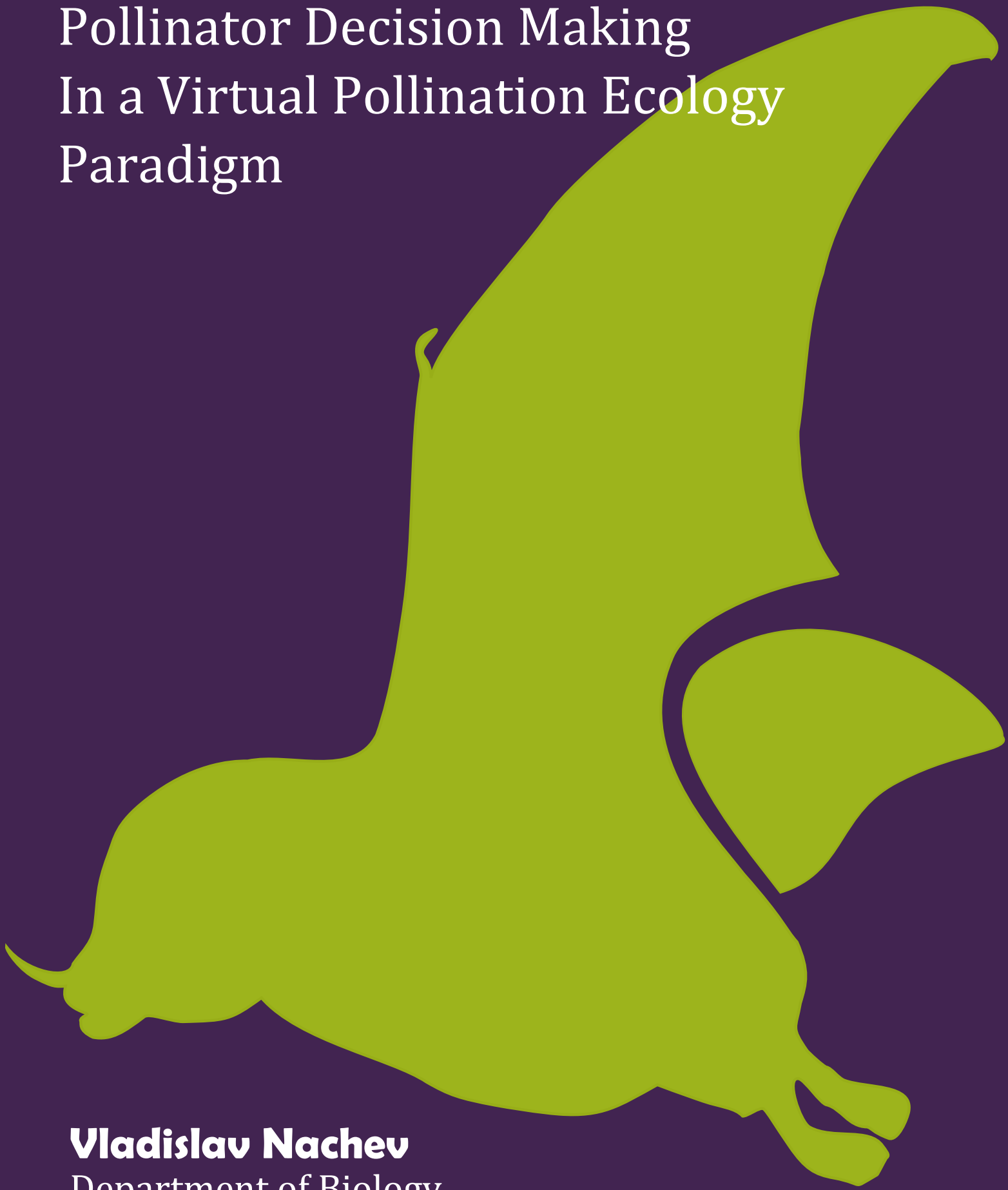
Cognition Mediated Floral Evolution:

Pollinator Decision Making
In a Virtual Pollination Ecology
Paradigm



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Cognition mediated floral evolution:

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Zusammenfassung

Von Schmetterlingen und Bienen bis Kolibris und Fledermäusen hat sich eine große Vielfalt von Tieren auf Blumennektar als Nahrung spezialisiert. Man unterscheidet dabei den Energie- und den Nährstoffbedarf verschiedener Bestäuber. Die Nektareigenschaften der vielen Pflanzenarten scheinen den Bedarf des Hauptbestäubers widerzuspiegeln, z.B. produzieren die von größeren Tieren bestäubten Pflanzen in der Regel auch größere Mengen an Nektar. Diese Übereinstimmung deutet darauf hin, dass Nektarmerkmale in Erwiderung auf die Auswahlkriterien der Bestäuber evolviert sind. Da Nektar eine unregelmäßig verteilte, ortsfeste, und erneuerbare Nahrungsquelle darstellt, können Bestäuber nicht nur mehrmals mit unterschiedlichen Pflanzen einer Art interagieren, sondern auch mit demselben Pflanzenindividuum – Bedingungen also, die das Lernen ermöglichen. Somit hängt die evolutionäre und ökologische Interaktion zwischen Pflanze und ihrem Bestäuber in entscheidender Weise von dessen Fähigkeit ab Unterschiede bei den Pflanzenmerkmalen wahrzunehmen, und von den Mechanismen der Entscheidungsfindung.

In der vorliegenden Arbeit steht die Ökologie kognitiver Funktionen im Vordergrund, um die Rolle der Informationsverarbeitung bei Bestäubern für die Evolution von Blütennektarmerkmalen zu untersuchen. In den ersten drei Kapiteln konzentriere ich mich auf die Fähigkeiten verschiedener Bestäuber zwischen Zuckerlösungen mit unterschiedlichen Konzentrationen zu diskriminieren. Im ersten Kapitel wird die psychometrische Analyse des Wahlverhaltens freifliegender wilder Fledermäuse (*Glossophaga commissarisi*) dargestellt. Es wurden psychometrische Funktionen angepasst, die die Stärke der Präferenz für die Optionen mit höherer Konzentration in Relation zur relativen Intensität der präsentierten Stimuli setzen. Im zweiten Kapitel wende ich die gleiche psychometrische Analyse auf freifliegende Hummeln (*Bombus impatiens*) an. Sowohl die Fledermäuse als auch die Hummeln bevorzugten die süßeren Optionen bei höheren relativen Intensitätswerten, zeigten hingegen jedoch keine Präferenz bei niedrigeren Intensitätswerten. Der Vergleich zwischen den Parametern der psychometrischen Funktionen der zwei Tierarten weist darauf hin, dass Fledermäuse einen geringeren Selektionsdruck auf höhere Nektarkonzentration als Hummeln ausüben. Dieses Ergebnis steht im Einklang mit der Beobachtung, dass die von Fledermäusen bestäubten Pflanzen in der Regel verdünnteren Nektar haben als die von Bienen bestäubten Pflanzen.

Die Ergebnisse der ersten beiden Studien deuten auf ein generelles Muster hin: Süßere Lösungen werden bevorzugt und bei niedrigeren Konzentrationen wird präziser diskriminiert. Zumindest qualitativ steht dieses Muster im Einklang mit dem Weberschen Gesetz, welches besagt, dass der empfundene Unterschied zwischen Stimuli vom Mittelwert ihres Ausmaßes abhängt. Im dritten Kapitel wird eine Laborstudie mit blütenbesuchenden Fledermäusen (*Glossophaga soricina*) dargestellt, die darauf abzielt die Genauigkeit der vom Weberschen Gesetz hergeleiteten Vorhersagen des Wahlverhaltens zu überprüfen. Die Fledermäuse zeigten eine geringere Diskriminationsleistung als vom Weberschen Gesetzt vorhergesagt. Eine bessere Anpassung wurde mit einem generalisierten Modell, dem so genannten „Near miss to Weber's law" erreicht. Eine zusammenfassende Betrachtung der Daten aus Kapitel 1 und 2 mit publizierten Daten von Kolibris und Honigbienen zeigt, dass das „Near miss to Weber's law" insgesamt eine bessere Anpassung bei allen Gruppen nektarfressender Tieren liefert. Diese Ergebnisse weisen auch darauf hin, dass Insekten zumindest bei der Wahl zwischen Optionen mit insgesamt hohen Zuckerkonzentrationen bessere Diskriminationsleistungen zeigen, als Wirbeltiere. Diese Schlussfolgerung passt zu der Beobachtung, dass die von Insekten bestäubten Pflanzen typischerweise süßeren Nektar produzieren, als die von Wirbeltieren bestäubten Pflanzen.

Bei allen bisher erwähnten Studien wurden zum Teil beträchtliche Unterschiede im Diskriminationsvermögen zwischen einzelnen Individuen beobachtet. Im vierten Kapitel werden solche individuellen Unterschiede auch auf der Ebene des Nahrungssuchverhaltens genauer analysiert und mit der Effizienz des Nahrungssuchverhaltens in Zusammenhang gebracht. Anhand der von *Glossophaga commissarisi* gewonnenen Felddaten wird der Zusammenhang zwischen der Effizienz der Nahrungsaufnahme und anderen Parametern des Nahrungssuchverhaltens untersucht, z. B. Flugaktivität und Anzahl der verschiedenen ausgebeuteten Blüten. Die Ergebnisse zeigten, dass Fledermäuse, die nur wenige Kunstblüten besuchten, in dem experimentellen Kontext effizienter Nahrung suchten als Fledermäuse, die eine größere Anzahl an Kunstblüten aufgesucht hatten. Dieser Befund stützt die Hypothese, dass individuelle Verhaltensunterschiede, auch als „Tierpersönlichkeiten" bezeichnet, letztlich zu Fitnessunterschieden führen können, was aber wiederum von den spezifischen ökologischen Umständen abhängig ist.

Das fünfte und letzte Kapitel baut auf den gewonnenen Erkenntnissen zur Psychometrie der Nektarqualitätswahrnehmung auf und befasst sich mit der evolutionären Entstehung von Nektareigenschaften. Es behandelt insbesondere das Phänomen der

niedrigeren Nektarkonzentration bei von Fledermäusen bestäubten Pflanzen, deren Vorfahren zum Teil über hohe Nektarkonzentrationen verfügten. Dieser Übergang ist ein evolutionäres Rätsel, denn Fledermäuse bevorzugen in Wahlsituationen normalerweise süßeren Nektar. Hierfür ist ein neues experimentelles Verfahren der virtuellen Evolution entwickelt und angewendet worden, bei dem Bestäuber Selektion auf virtuelle Pflanzen ausüben. Aus der Kombination von Labor- und Felddaten zusammen mit den Ergebnissen von theoretischen Modellen lässt sich erklären, wie die Evolution in Richtung niedrigerer Nahrungsqualität stattfinden kann. Die Ursache liegt in dem Entscheidungsverhalten, welches der Bewertung von Nahrungsqualität und Nahrungsmenge folgt. Wenn die Bewertung dieser Belohnungsparameter nichtlinear ist und außerdem die verfügbaren Entscheidungsoptionen sich in ihrer Varianz unterscheiden, dann sagt auch die theoretische Analyse die experimentell beobachtete Evolution hin zu niedrigen Zuckerkonzentrationen im Nektar voraus. Aus mehr allgemeiner Sicht verdeutlichen diese Ergebnisse auch, wie der „Preis“ für ein bestimmtes Bedarfsobjekt in einem hoch dynamischen System in Reaktion auf wachsende Nachfrage evolviert. Die Ergebnisse lassen sich möglicherweise auch verallgemeinern um die Evolution der Nektarkonzentration bei anderen Pflanzen zu erklären, die ihre Hauptbestäuber wechseln.

Diese Studien zeigen, wie die Untersuchung kognitiver Mechanismen von Bestäubern die evolutionäre und ökologische Forschung an zoophilen Pflanzen voranbringen kann. Zusätzlich wird somit Folgendes aufgewiesen: Der Methodenansatz der virtuellen Bestäubungsökologie kann aussagekräftige Erklärungen liefern für die evolutionäre Entstehung sowie Aufrechterhaltung von Pflanzenmerkmalen, die einer durch Kognition vermittelten und von Bestäubern ausgeübten Selektion unterliegen.

Summary

A diverse array of animals has specialized in consuming floral nectar – from butterflies and bees to hummingbirds and bats. The energetic and nutritional needs differ among pollinators and the nectar characteristics of plant species often appear to reflect the needs of their dominant pollinator, for example plants pollinated by larger animals tend to produce larger amounts of nectar. This correspondence suggests that nectar traits have evolved in response to the choice behavior of pollinators. As nectar is a patchily distributed, stationary, and renewable food source, pollinators often repeatedly interact not only with plants from the same species, but with the same plant individuals, providing an opportunity for learning. Thus, the evolutionary and ecological interaction between plants and their pollinators crucially depends on the pollinators' ability to perceive differences in floral nectar traits and on their decision-making mechanisms.

In the presented studies I adopt a cognitive ecology approach in order to investigate the role of information-processing in pollinators on the evolution of floral nectar traits. In the first three chapters I focus on the abilities of different pollinators to discriminate among sugar solutions with different concentrations. In Chapter 1 I present a psychometric analysis of the choice behavior of free-flying wild glossophagine bats (*Glossophaga commissarisi*). I fit individual psychometric functions, relating the strength of preference for the higher concentration option to the relative intensity of the presented stimuli. In Chapter 2 I performed the same type of psychometric analysis on free-flying bumblebee workers (*Bombus impatiens*). Both bats and bumblebees preferred the sweeter options at high relative intensities and showed no preference at low intensities. A comparison of the psychometric function parameters between the two species suggests that bats exert a weaker selection pressure for higher sugar concentrations than bees. This result is consistent with the observation that bat-pollinated plants tend to have more dilute nectars than bee-pollinated plants.

The results from these two experiments suggest a general pattern of preference for sweeter sugar solutions and more precise discrimination at low concentrations. At least qualitatively, this pattern is consistent with Weber's law, which states that the perceived difference between stimuli depends on their mean magnitude. In Chapter 3 I present a laboratory study with nectar-feeding bats (*Glossophaga soricina*), in which I investigate whether Weber's law accurately predicts choice behavior. Bats showed a lower discrimination

performance than predicted by Weber's law and a better fit was achieved with a more generalized model, the so-called "near miss to Weber's law". A reanalysis of the data from Chapters 1–2, along with previously published data on hummingbirds and honeybees, revealed that the near miss to Weber's law provides a better fit in all groups of nectar-feeding animals. The results also suggest that, at least when choosing between options with high concentrations, insects show better discrimination performances than vertebrates. Again, these findings are consistent with the observation that insect-pollinated plants typically produce sweeter nectars than vertebrate-pollinated plants.

In all studies mentioned so far there were considerable differences in discrimination performance among individuals. In Chapter 4 I present a detailed analysis of such individual differences in the foraging context and discuss how they might relate to foraging efficiency. I used the *G. commissarisi* field data set to investigate the link between foraging performance and other measures of foraging behavior, such as flight activity and number of different flowers exploited. I found that bats that visited fewer feeders exhibited more efficient foraging within the experimental context than bats that visited more different feeders. This finding supports the hypothesis that individual behavioral differences, often referred to as "animal personalities", can result in differences in fitness, depending on the particular ecological conditions.

In the fifth and final chapter I use the findings on the psychophysics of nectar quality evaluation to address the question of the evolutionary origins of floral nectar traits. In particular, I focus on the peculiar observation that bat-pollinated plants have low nectar concentrations despite being descended from at least some putative ancestors with high nectar concentrations. This transition constitutes an evolutionary puzzle, because bats usually prefer sweeter nectars when choosing freely. For this research project I developed and applied a novel experimental approach called virtual pollination ecology, in which pollinators exert selection on virtual plants. I present laboratory and field data, along with theoretical models that explain how the evolution towards lower food quality can be triggered. The answer lies in the choice behavior of pollinators, which depends on the evaluation of both food quality and food amount. My theoretical analysis predicts that, if the evaluation of these two reward parameters is non-linear and if the available options differ in their variance, selection favors lower sugar concentrations, just as was observed in the virtual evolution experiments. From a broader perspective my results also illustrate how the "price" of a commodity in a highly-dynamic system evolves in response to increasing demand. The results can be potentially

generalized to explain the evolution of nectar concentrations in other plants that experience pollinator shifts.

With these studies I show how the investigation of cognitive mechanisms of pollinators can inform evolutionary and ecological research on plants pollinated by animals. In addition, I demonstrate how the virtual pollination ecology methodology can explain the evolutionary origin and maintenance of plant traits that are subjected to cognition-mediated selection exerted by pollinators.

CHAPTER 1: The psychophysics of uneconomical choice: non-linear reward evaluation by a nectar feeder

Abstract. Uneconomical choices by humans or animals that evaluate reward options challenge the expectation that decision-makers always maximize the return currency. One possible explanation for such deviations from optimality is that the ability to sense differences in physical value between available alternatives is constrained by the sensory and cognitive processes for encoding profitability. In this study, I investigated the capacity of a nectarivorous bat species (*Glossophaga commissarisi*) to discriminate between sugar solutions with different concentrations. I conducted a two-alternative free-choice experiment on a population of wild electronically tagged bats foraging at an array of computer-automated artificial flowers that recorded individual choices. I used a Bayesian approach to fit individual psychometric functions, relating the strength of preferring the higher concentration option to the relative intensity of the presented stimulus. Psychometric analysis revealed that discrimination ability increases non-linearly with respect to relative intensity. I combined this result with a previous psychometric analysis of volume perception. My theoretical analysis of choice for rewards that vary in two quality dimensions revealed regions of parameter combinations where uneconomic choice is expected. Discrimination ability may be constrained by non-linear perceptual and cognitive encoding processes that result in uneconomical choice.

Introduction

Value-based decision making requires that the value of an option can be sensed and stored in memory. This makes a comparison between options possible. Decision-making processes can sometimes lead to outcomes that are not economical (Livnat and Pippenger 2008). This is the case when the decision-maker prefers an option with a lower caloric return over another with a higher caloric return, or when it fails to discriminate between options with different caloric contents. In order to understand how underlying mechanisms can lead to uneconomical choices it is necessary to have a quantitative understanding of the steps involved in the evaluation process. Sensing the caloric value of a reward, and being able to discriminate between rewards, is the first step in such a process and the objective of this study.

Such considerations about value-based decision making are also relevant for understanding the co-evolutionary development of energy rewards offered by plants to their pollinators. This is relevant in the context of this study, in which I investigated choice behavior of nectar-feeding bats. The evolution of flower traits in animal-pollinated plants is shaped in part by the selection pressure to offer attractive energy resources to potential pollen vectors (Zimmerman 1983; Real and Rathcke 1991; Sakai 1993). Among pollinator attractants, the most common are simple carbohydrates presented as nectars, i.e. sugar-water solutions (Baker and Baker 1983; Stiles and Freeman 1993). From the pollinators' perspective, nectars with higher sugar concentrations represent richer energy sources that should be preferred by foragers seeking to optimize their energetic gains. Diverse groups of nectar-feeding animals such as bees (e.g. von Frisch 1927; Cnaani et al. 2006), birds (e.g. Hainsworth and Wolf 1976; Roberts 1996; Nicolson and Fleming 2003) and bats (e.g. Roces et al. 1993; Rodríguez-Peña et al. 2007) show a general pattern of preference for sweeter sugar solutions and more precise discrimination at low concentrations. In the case of nectar-feeding (glossophagine) bats, preference has been estimated by measuring differences in bat visitation and consumption rates at food sources with differing nectar qualities. However, our knowledge on the ability of bats to discriminate between nectar concentrations remains fragmentary and inconclusive, even though this ability has direct consequences on decision making and on the selection pressures exerted by the bats.

A standardized method for estimating discrimination ability is fitting a psychometric function to data from alternative forced-choice tasks (Treutwein and Strasburger 1999). The psychometric function relates the behavioral response of the animal to the intensities of the physical stimuli. This methodology has been applied to estimate the ability of the flower-

visiting and nectar-feeding bat *Glossophaga soricina* to discriminate between two volumes (Toelch and Winter 2007). Measuring the volume of nectar obtained from a feeding event is one necessary component for reward evaluation. With this study I determined the second necessary component for a nectar-feeding animal: the ability to evaluate the concentration of sugar in a nectar reward. For this I performed a psychometric analysis of concentration discrimination ability in a nectarivorous bat species. I obtained data from a two-alternative free-choice test performed with a group of wild, free-flying *Glossophaga commissarisi* bats.

Methods

Study Site and Subjects

Experiments were conducted from February to April 2009, at La Selva Biological Station, Province Heredia, Costa Rica. Wild bats were initially recruited to visit the experimental site by setting up nectar feeders filled with 20% sugar solution and equipped with a dimethyl disulphide reservoir giving off odor plumes to act as a far-range attractant to the bats (von Helversen et al. 2000). Feeders were mounted on an aluminum rectangular frame (2 × 4 m), suspended below a 3 × 6-m steel frame canopy, which provided cover from the rain. The frame was parallel to and 1.6 m above the ground. Using mist-nets I caught and marked 63 adult individuals, 39 males and 24 females, of the common (Tschapka 1998) nectarivore *Glossophaga commissarisi* Gardner. Bats were weighed, sexed, marked with RFID collars, and released at the site of capture. Over the course of the study, 54 of these bats were registered visiting the artificial flowers, along with an unknown number of unmarked bats and other visitors. Permission for experimentation and RFID-tagging was obtained from Sistema Nacional de Areas de Conservación (SINAC) at the Ministerio de Ambiente y Energía (MINAE).

Artificial Flowers

For the purpose of this study, a model was developed that incorporates some characteristics of typical Neotropical chiropterophilic plants, e.g. the bromeliad *Werauhia gladioliflora*, a common bat-pollinated plant in the area of this study (Tschapka and von Helversen 2007). Individual plants were represented by computer-controlled feeders (Winter and Stich 2005; Santoso et al. 2006; Thiele 2006), which delivered sugar water (hereafter

"nectar"). I used twenty-four feeders mounted under the steel frame canopy. The distance between flower "corollas" in the same row was about 40 cm and the distance between rows about 60 cm. Such plant density is not unusual for *W. gladioliflora* (personal observation). The control computer, hardware interface, power supply units, and nectar reservoirs were all placed in an air-conditioned shed, some 5 meters away from the canopy set-up and connected to it via signal cables, power leads, and main nectar tubes. Visits to the feeders were registered with an infrared beam detector, and transponder-reading devices identified individuals carrying RFID tags. Each feeder was equipped with two solenoid pinch valves and connected to two nectar delivery systems via tubing systems (Fig. 1). Nectar reward delivery was controlled by two syringe pumps using two gas-tight Hamilton glass syringes (Series 1025). Feeders delivered 55–60 µL rewards on every visit.

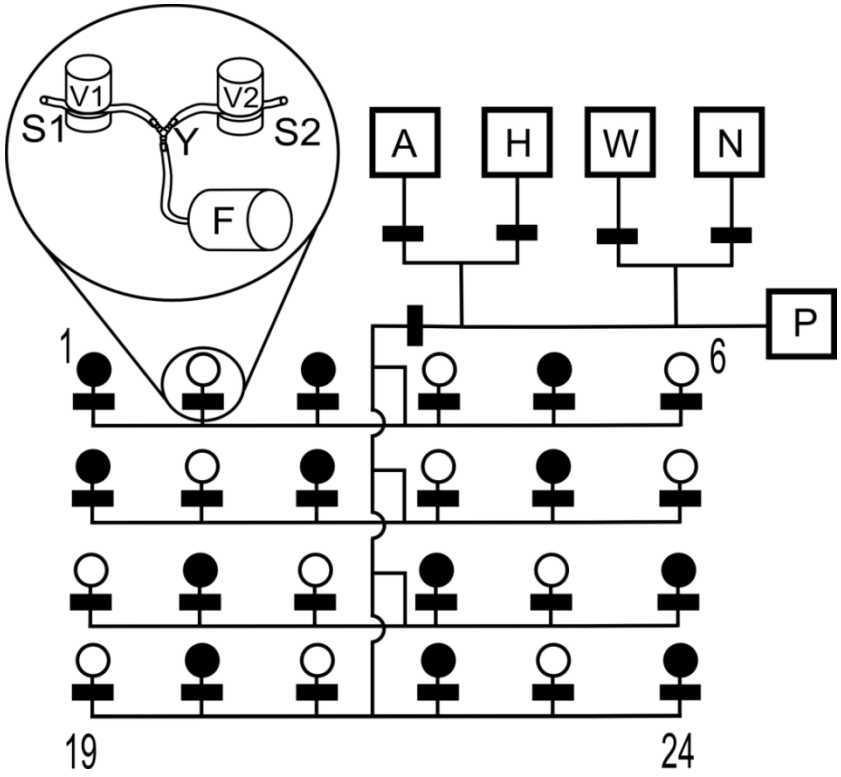

Figure 1. Pump and tubing system of the 24-feeder array**.** *Lines* represent the tubes, and *black rectangles* the pinch valves. Feeders are numbered *1–24*. *Boxes* represent the following liquid reservoirs: ethanol (*E*), water (*H*), waste (*W*), nectar (*N*), and stepping-motor syringe pump (*P*), as described in Winter and Stich (2005). Length of tubes not drawn to scale. Two identical tubing systems were connected to the feeders. The merging point of the tubing systems is illustrated in the *inset*: magnetic pinch valves for the first (*V1*) and second tubing systems (*V2*), with their corresponding tubes (*S1* and *S2*), a Y connector (*Y*) and feeder head (*F*). Feeders represented by *black circles* only received nectar from S1, and feeders shown in *white* were only fed by *S2*. The two pumping systems were filled with different sugar concentrations on different days. See "Methods" for further details

Nectar consisted of fructose and sucrose (2:1 parts) dissolved in water, with a hexose to sucrose ratio similar to the ratio in natural nectars of glossophagine-pollinated plants (Baker et al. 1998). Half of the feeders received nectar from one pumping system, and the other half from the other system (Fig. 1). The two systems were filled with nectars with different concentrations. Thus during a single night the concentration offered at each feeder was fixed and did not change. In order to prevent bacterial and fungal growth inside the tubing systems, they were rinsed with water and a 70%–ethanol solution every 3–4 days.

Experimental Schedule

I recorded data between 18:00 and 06:00 h. The nectar concentrations ranged from 5 to 50% weight/weight (or 148 to 1796 mmol L^{-1} sucrose equivalents, Bolten et al. 1979) and were presented in two series of two-alternative free-choice tests, with 12 feeders per option. The first series of tests consisted of nine different conditions with a difference between the two options of 5% (from 5% vs. 10% to 45% vs. 50%). The second series consisted of seven conditions with a difference of 15% (from 5% vs. 20% to 35% vs. 50%) between options. The sequence of conditions within both series was random. However, every condition was presented twice on consecutive nights on which the feeder positions for each concentration were exchanged (Fig. 1, black and white feeders), as a control for positional biases. The choice of pumping system for the higher nectar concentration during the first night of each condition was random.

Data Analysis

Recorded data consisted of the time-stamped visitation events of marked bats and unknown unmarked visitors. Analysis was limited to the hours between 20:00 and 03:00 h. I excluded the hours before 20:00 h in order to focus on plateau performance, after the initial sampling and exploration phase. The visits after 03:00 h were excluded because of unexpectedly high visit numbers and premature depletion of the nectar supply on some nights. (This only occurred during two nights in the 5% series and three nights in the 15% series.) For each bat and each condition, I calculated the *relative intensity* and the *discrimination performance*. The *relative intensity* was calculated as the absolute difference between the two

sugar concentrations, divided by the mean concentration. Over the two presentations of the same condition, *discrimination performance* was calculated as the number of visits to higher sugar concentration feeders divided by the total number of visits. If a bat showed a perfect "preference" for one feeder type without having made any visits to the other type during a whole night, including the time before 20:00 h, its data for that night were eliminated from the analysis.

Psychometric Analysis

I performed individual psychometric analyses on the data from each animal and fitted Weibull psychometric functions using the algorithm proposed by Kuss et al. (2005) using R 2.10.1 (R Development Core Team 2009). A similar application of this method is presented in Toelch and Winter (2007). In this Bayesian approach, Markov Chain Monte Carlo (MCMC) sampling is used to estimate the threshold, slope, and lapse rate of the psychometric functions, along with their confidence intervals. The point on the curve halfway between the lower and upper asymptote (corresponding to a discrimination performance of ca. 75%) is referred to as the threshold. The slope of the function at the threshold is interpreted as a reliability measure of sensory performance (Treutwein and Strasburger 1999). Finally, the lapse rate is a measure of the frequency of errors (in this case, visits to the low concentration feeders) due to distraction, motivational problems, and other factors of a non-perceptual nature. In this particular application, it may also be interpreted as a base rate of exploration. As prior function for the lapse rate, we chose a beta distribution (2; 50). For the threshold we chose a normally distributed prior with a mean of 1 and a standard deviation of 0.5, and for the slope a log-normal prior with a mean of 2 and a standard deviation of 1. We performed 5,000 MCMC sampling runs with a leapfrog step size of 100. From the individual psychometric functions obtained using this method, we calculated the mean and 95% confidence intervals for the threshold, slope, and lapse rate. Researchers have shown that the fit of the psychometric function is very sensitive to the sampling scheme, i.e., the choice of stimulus intensities and their distribution (Wichmann and Hill 2001). The intensities resulting from the chosen sugar concentrations in the 5% series were clustered in a region of lower to medium intensities without critical values around the threshold. On the other hand, the intensities resulting from the 15% series resulted in a wider range of intensities and included two points around the threshold. The theoretical expectation was that relative differences rather than absolute differences would predict bat choice. This relative difference is captured by the relative intensity measure expressed in terms of the absolute difference divided by the mean stimulus

magnitude. I therefore restricted individual psychometric analysis to the 23 most active bats, which made on average more than 50 visits per night during the 15% series and were absent for no more than a single test condition from that series. With the exception of three bats, these 23 animals were also detected during the 5% series and analysis was performed on the pooled data from both series. From the individually fitted psychometric functions, I calculated the mean of the lapse rate, threshold, and slope across animals. For visualization purposes, I also fitted a psychometric function on the pooled data from all 23 bats.

Results

Between 20:00 and 03:00 h, the bats selected for analysis made an average of 75 visits per bat per night (excluding bats which made no visits, SD = 58, N = 23 bats). These visits represented 26% of the total registered visits between 20:00 and 03:00 h. Discrimination performance decreased with increasing average concentration of the presented stimuli in both experimental series (Fig. 2a). Bats showed no discrimination between concentrations at low relative intensities and good discrimination at high relative intensities (Fig. 2b). As a reminder, the lowest relative intensity presented was 45% versus 50% and the highest relative intensity was 5% versus 20%. The average threshold (ca. 75% discrimination performance) of the psychometric functions calculated for the individual bats was 0.50 ± 0.073 SD, N = 23. The average lapse rate and slope were 0.04 ± 0.023 SD, N = 23, and 3.41 ± 1.34 SD, N = 23, respectively. Restricting the analysis only to the animals that were present during every single night (N = 6) produced similar results: the values for the threshold, lapse rate, and slope were 0.50 ± 0.049 SD, 0.04 ± 0.025 SD, and 2.66 ± 0.93 SD, respectively. Fitting a psychometric function to the pooled data from all visitors, including unmarked animals, also produced similar results: the values for the threshold, lapse rate, and slope were (mean ± SD) 0.52 ± 0.002, 0.06 ± 0.002, and 2.02 ± 0.03, respectively, (N = 5,000 MCMC simulation runs).

Discussion

The ability of *G. commissarisi* to discriminate between sugar concentrations can be described with the psychometric function presented in this study (Fig. 2). Within the tested range of concentration pairs, bats either made more visits to the feeder with more concentrated nectar

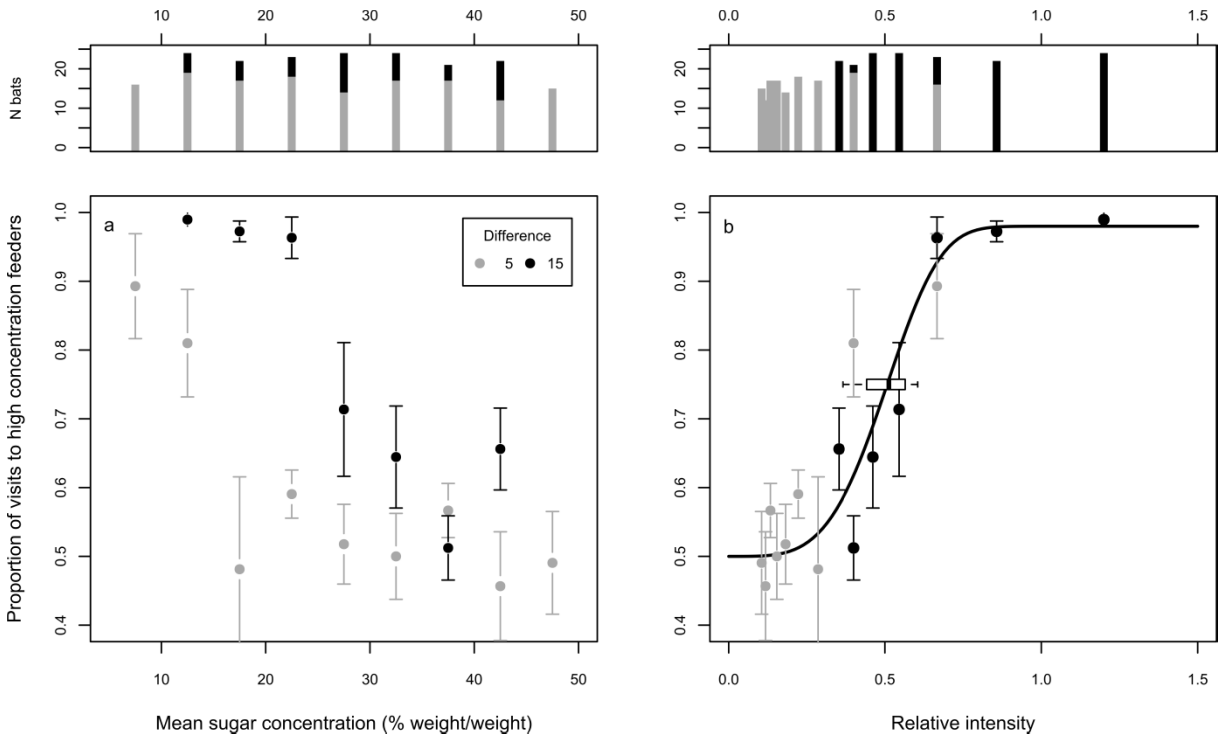

Figure 2. Bat visitation patterns to feeders of different concentrations. **a** Relative visitation rate versus mean sugar concentration. *Circles* represent the median proportion of visits to higher concentration feeders for the series with 5% difference (*gray*) and with 15% difference (*black*). *Upper panel* shows the number of bats in the tested conditions for the 5% difference (*gray*) and 15% difference series (*black*). *Error bars* give the median absolute deviation calculated over the respective number of bats. **b** Psychometric curve for concentration discrimination. Same data and symbols as in **a**, but with relative intensities on the *abscissa* (for relative intensity calculations, see "Methods"). The *line* represents the psychometric function fitted to the pooled data. The *box* gives the median (here at 0.5) and 95% confidence interval of the threshold values (75% discrimination performance) calculated for the individual bats

or showed no preference. The psychometric function can also be used to predict psychometric discrimination performance of bats for any pair of sugar concentrations between 5% and 50%. For example, for relative intensities higher than the threshold ($x > 0.5$), the psychometric function predicts that the options with the more concentrated nectars will receive at least 75%

$$\Psi(x,m,s,\pi_l) = \frac{1}{2}\left[\pi_l + (1-\pi_l)\left[2 - \exp\left(-\exp\left(\frac{2sm}{\ln(2)}\left(\ln(x)-\ln(m)\right)+\ln\left(\ln(2)\right)\right)\right)\right]\right] \quad (1)$$

of all visits. The predicted relative visitation rate to the sweeter option of two concentrations with relative intensity x can be calculated with the following equation:

where m is the threshold, s is the slope at the threshold, and π_l is the lapse rate (from equations (1) and (11) in Kuss et al. 2005). If we have a given standard concentration c_2 and we want to obtain the concentration c_1 ($c_1 > c_2$) that paired with the standard will result in discrimination at some intensity level i, we can use the formula for intensity calculation to obtain the following ratio, which is constant for any chosen i:

$$\frac{c_1}{c_2} = \frac{2+i}{2-i}. \tag{2}$$

The ratio of the difference of the two concentrations and the standard is also constant:

$$\frac{c_1 - c_2}{c_2} = \frac{2i}{2-i}. \tag{3}$$

My results indicate that the evaluation of sugar concentrations by *G. commissarisi* is affected by two systematic biases. Discrimination performance improves as the difference between alternative choices increases (distance effect). Discrimination performance declines as distance (the absolute difference between two concentrations) is kept constant but the average concentration of the two options increases (magnitude effect). Stimulus comparison in the case of sugar concentrations cannot occur simultaneously. Instead, the currently experienced concentration must be compared with a sample retrieved from memory. Presumably the distance and magnitude effects are consequences of sensory transfer functions and the memory representation of sweetness, or caloric value. In essence, these effects and Eq. 3 above are consistent with Weber-Fechner's law, which states that physical stimuli are scaled on a logarithmic internal representation over a major part of their perceptible range and that a differential threshold such as the *just-noticeable difference* (*jnd*) is a constant fraction of the magnitude of the stimulus (Kacelnik and Brito e Abreu 1998; Deco and Rolls 2006; Kang et al. 2010). I suggest that a non-linear perception and encoding process can also explain the biases described in this study.

The capacity of *G. commissarisi* to discriminate between nectar concentrations appears to be very similar to that of congeneric *G. soricina* (Fig. 3). In contrast, data obtained from the larger, more specialized nectarivore *Leptonycteris yerbabuenae* (formerly *L. curasoae*) imply that its psychometric function has a lower threshold compared with the two *Glossophaga* species (Fig. 3). This may indicate a general trend among phyllostomids that the degree of diet specialization on sugar-rich flower nectar will negatively correlate with the psychometric function threshold. Such an evolutionary trend could be driven by the costs associated with sensory processing and the resulting energy-information trade-off (Isler and van Schaik 2006; Niven et al. 2007; Niven and Laughlin 2008). Increasing signal-to-noise-

ratio or bandwidth causes disproportionate increases in energetic cost at the cellular level which in turn constitutes a severe penalty on excess functional capacity (Niven et al. 2007; Niven and Laughlin 2008). Furthermore, even a theoretically optimal decision-maker produced by natural selection is still expected to make systematic mistakes (Livnat and Pippenger 2008).

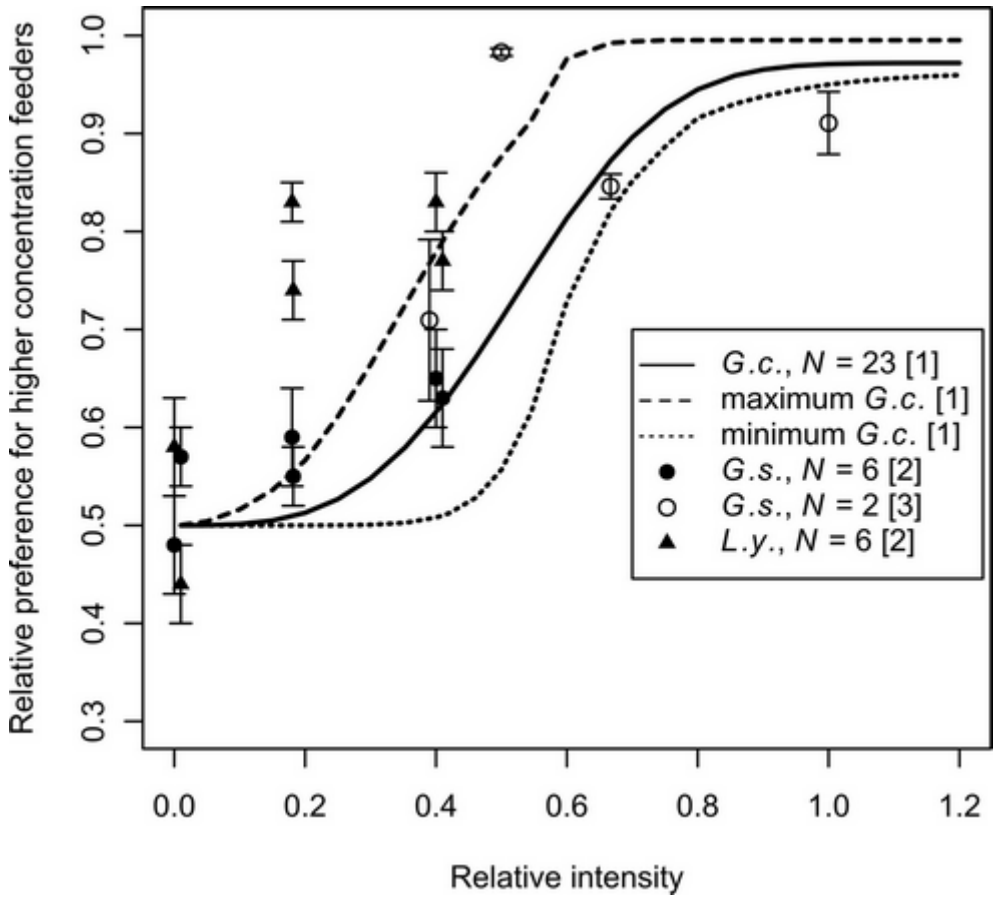

Figure 3. Concentration discrimination in three different glossophagine bat species. Relative intensities are given on the *abscissa* (for relative intensity calculations see "Methods"). The *continuous line* is the psychometric function estimated from the mean threshold, slope and lapse rate of the individuals tested in this study. The *dashed lines* represent the maximum (*longer dashes*) and minimum (*shorter dashes*) values from the individually fitted psychometric functions of the 23 animals tested in this study. *Symbols* represent mean preferences for the more concentrated feeder type and *whiskers* represent one standard error. For clarity in the graph, horizontal jitter of 0.1 is added to points with the same relative intensity in order to prevent overlap. *G.c.* = G*lossophaga commissarisi*, *G.s.* = *G. soricina*, *L.y.* = *Leptonycteris yerbabuenae*. Sources: [1] = This study; [2] = Rodríguez-Peña et al. (2007); [3] = Roces et al. (1993)

We now have available the two psychometric functions that describe the perception of sugar concentration and nectar volume in *Glossophaga*. These functions can serve as the basis

for any decision that evaluates nectar reward quality. The mathematical nature of the psychometric functions makes it possible to predict theoretically the parameter space where uneconomical choice is expected. For the following, I first assume that the psychometric function for volume perception estimated in *G. soricina* has the same parameters (threshold = 0.75, lapse rate = 0.05, slope = 1.6, average values from Table 2 in Toelch and Winter 2007) in *G. commissarisi*. Second, I assume equal lapse rates for both functions at 0.05. Finally, I assume that when the psychometric functions for concentration and volume predict different choices, choice is determined by the reward dimension predicting the higher relative visitation rate. In case of a tie, the opposite predictions neutralize each other and choice becomes random. Consider for example a reference reward type with 20% concentration and 30 μL volume. The predicted relative preference for alternative options with the same volume as the reference and different concentrations is given in Fig. 4a. The predicted relative preference for alternative options with the same concentration as the reference and different volumes is given in Fig. 4b. The gray areas in Fig. 4c indicate the combinations of volumes and concentrations that – paired with the reference – are predicted to result in non-profitable choices.

Food choice experiments in which both volume and concentration are manipulated indicate, as one would expect, that nectar-feeding animals estimate sugar concentration and nectar volume using different mechanisms, rather than evaluating overall sugar intake over time (Bateson et al. 2003; Cnaani et al. 2006). In these two studies, animals were presented with equicaloric options differing in volume and concentration. However, contrary to expectations of equal preference, animals made more visits to the options with the higher concentration. Such preferences, which remain to be tested in *Glossophaga*, could also be explained by corresponding psychometric functions for volume and concentration if for hummingbirds and bumblebees the mechanisms discussed here also apply. If the functions for volume and concentration discrimination are recalculated in Joules then the function for concentration has a threshold of 0.55, which is lower than the threshold for volume at 0.75. That means that for equivalent changes in caloric value bats are predicted to be more sensitive to changes in concentration than to changes in volume (Figure 5). Deviations from optimality can be even stronger if evaluation of reward properties takes place sequentially and if one dimension is given priority over the others. For example, Cnaani and colleagues (2006) suggest that bumble bees perceive sugar concentration first, and may reject a nectar reward if it is too dilute without consuming it completely and obtaining information about the available volume. Similarly, an emptied flower provides no information about its nectar concentration. Since

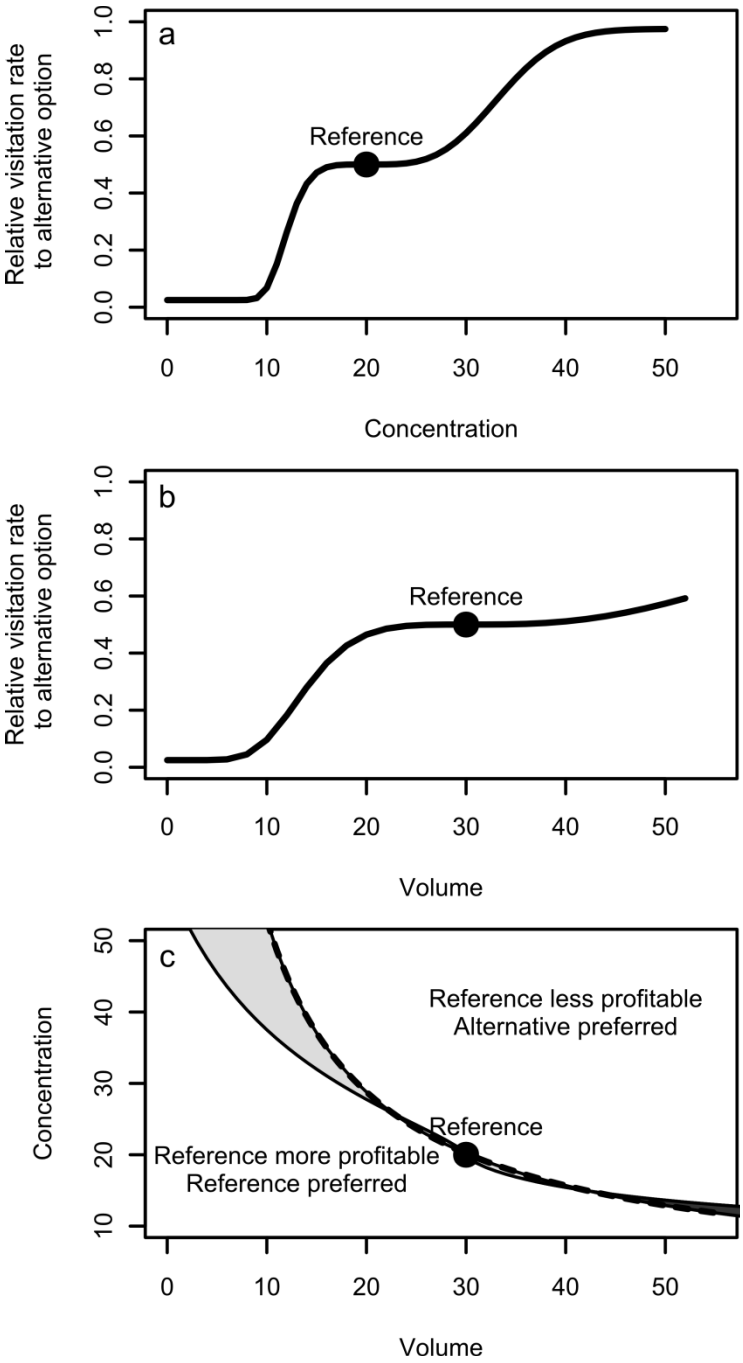

Figure 4. Relative preference for nectar sources in a choice between a reference option and alternatives with different volumes and concentrations. The reference option (*point*, all *panels*) has 20% concentration and 30 µL volume.

a Relative visitation rates to alternative options with different concentrations and volumes equal to the reference.

b Relative visitation rates to alternative options with different volumes and concentrations equal to the reference. The flat portion of the curves around the reference (**a**, **b**) is an "indifference zone" and indicates options for which bats are indifferent with respect to the reference. Preference increases more slowly for values higher than the reference (slope of curve to the right of the indifference zone) than it decreases for values lower than the reference (slope of curve to the left of the indifference zone). Curves in **a** and **b** calculated with Eq. 1.

c Preference for less profitable nectar sources when both volume and concentration are changed in the alternative options. Points on the *dashed line* have the same energetic value (Bolten et al. 1979) as the reference. Options above the *dashed line* have higher and below the *line* lower energetic values than the reference. The *continuous line* marks the options for which bats are indifferent with respect to the reference, based on psychometric predictions from Eq. 1. The *light gray area* between the two *curves* contains the options that are less profitable and preferred over the reference. The *dark gray area* denotes the cases in which the reference is preferred even though it is less profitable than the alternative option

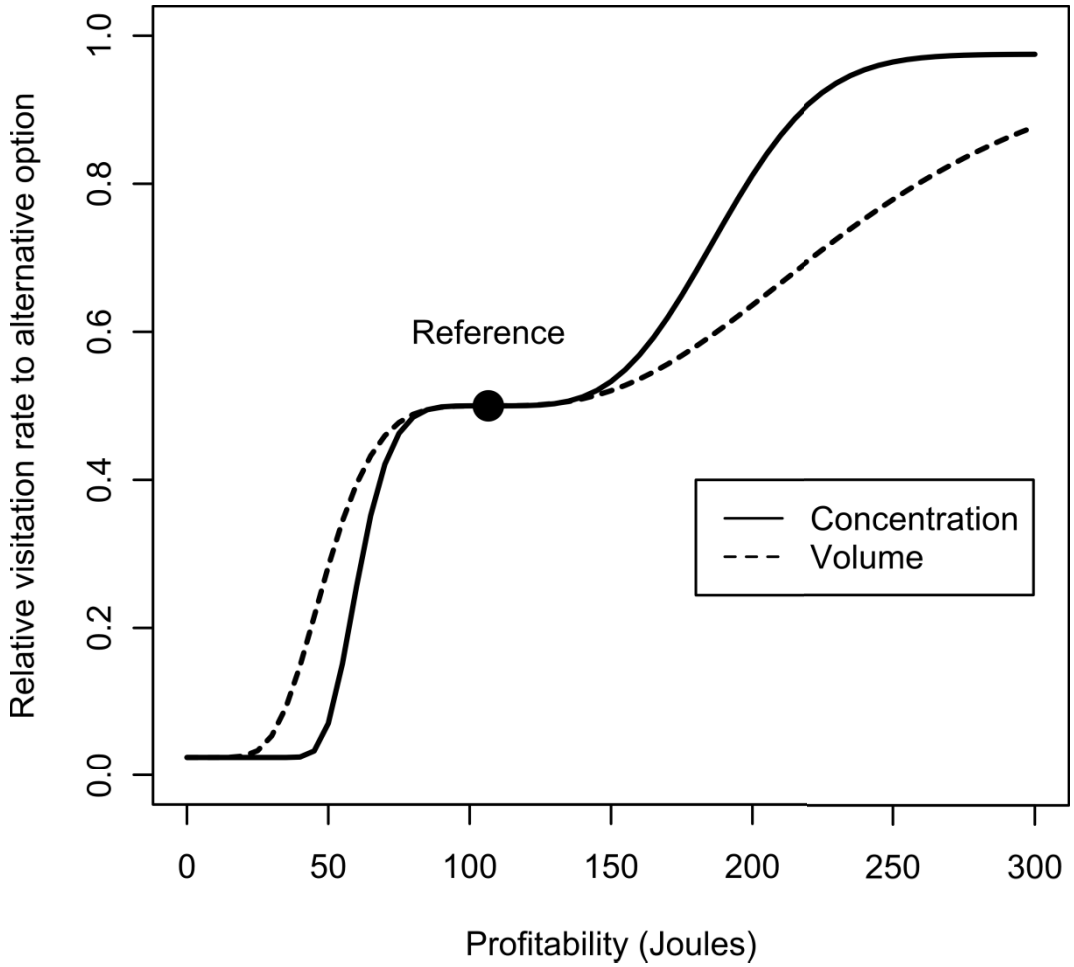

Figure 5. Psychometric functions for discrimination of sugar concentration (Fig. 4a) and nectar volume (Fig. 4b) recalculated in Joules. Although the two curves have roughly equal indifference portions, the concentration curve is steeper and has a lower threshold than the volume curve. Consequently, for sufficiently large changes in energetic content, bats are predicted to respond more strongly when the change is associated with a change in sugar concentration than with a change in volume. *Data* calculated with Eq. 1

bats' discrimination of feeders differing in their likelihood of being empty exhibits the same distance and magnitude effects described above (unpubl. data), variance-sensitive foraging behavior can also result in non-profitable choices. In summary, because of the shape of the psychometric functions, negative changes in nectar reward properties along one dimension, e.g. concentration, might be masked by positive changes along the remaining dimensions, reward probability and volume, even when these changes decrease overall profitability.

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CHAPTER 2: The psychophysics of sugar concentration discrimination and contrast evaluation in bumblebees

Abstract. The capacity to discriminate between choice options is crucial for a decision-maker to avoid unprofitable options. The physical properties of rewards are presumed to be represented on context-dependent, nonlinear cognitive scales that may systematically influence reward expectation and thus choice behavior. In this study, I investigated the discrimination performance of free-flying bumblebee workers (*Bombus impatiens*) in a choice between sucrose solutions with different concentrations. I conducted two-alternative free choice experiments on two *B. impatiens* colonies containing some electronically tagged bumblebees foraging at an array of computer-automated artificial flowers that recorded individual choices. I mimicked natural foraging conditions by allowing uncertainty in the probability of reward delivery while maintaining certainty in reward concentration. I used a Bayesian approach to fit psychometric functions, relating the strength of preference for the higher concentration option to the relative intensity of the presented stimuli. Psychometric analysis was performed on visitation data from individually marked bumblebees and pooled data from unmarked individuals. Bumblebees preferred the more concentrated sugar solutions at high stimulus intensities and showed no preference at low stimulus intensities. The obtained psychometric function is consistent with reward evaluation based on perceived concentration contrast between choices. I found no evidence that bumblebees reduce reward expectations upon experiencing non-rewarded visits. I compare psychometric function parameters between the bumblebee *B. impatiens* and the flower bat *Glossophaga commissarisi* and discuss the relevance of psychophysics for pollinator-exerted selection pressures on plants.

Introduction

Decision-makers such as foraging animals or humans choosing between gambles are able to utilize information about different parameters of the choice options (i.e. probability of reward, amount of reward: Markowitz 1952; Kahneman and Tversky 1979; Wedell 1991; Kacelnik and Brito e Abreu 1998; Bateson et al. 2003; Cnaani et al. 2006; Bacon et al. 2011). Theoretical analyses of choice assume that different reward dimensions are integrated into some common currency, that is "utility" (Chib et al. 2009; Kenrick et al. 2009). It is further assumed that behaviors maximizing the return currency are associated with fitness benefits and are the products of natural selection (Ritchie 1990; Kenrick et al. 2009). Underlying the capacity to make choices that maximize profitability is the ability to sense and evaluate differences among alternative options (Kacelnik and Brito e Abreu 1998; Livnat and Pippenger 2008; Shafir et al. 2008). Profitability maximization in the case of sequential sampling of multiple options relies on sensation (converting a physical stimulus into a neuronal firing pattern), memory (maintaining a representation of a physical stimulus over a period of time), and decision making (comparing representations from different sources and performing a motor task based on the results of this comparison). Hereafter, I refer to the conjunction of these three processes as "information processing".

Since the inception of the field of psychophysics, researchers have been interested in the neural and cognitive representations of physical scales (Fechner 1860; Thurstone 1927; Stevens 1961). As direct observations and measurements of subjective sensations are not possible, scientists have instead focused on measuring behavioral output or neuronal activity. Psychometric analyses of scales such as sweetness, heaviness, brightness, and even abstract scales such as time and numerosity typically reveal a nonlinear correspondence between the original scale and the psychological scale (Stevens 1961, 1969; Perez and Waddington 1996; Dehaene 2003; Toelch and Winter 2007; Billock and Tsou 2011). The logarithmic or weak power law compression of sensory information typically observed may result from the tuning properties of sensory neurons (Dayan and Abbott 2001) and has furthermore been suggested not only for sensory traces, but also for reactivated memories as well (Gallistel and Gelman 2000; Nieder and Miller 2003; Papini and Pellegrini 2006). This type of representational mechanism is robust against errors and arguably superior to alternative mechanisms (Sinn 2003; Portugal and Svaiter 2010), but it can influence choice behavior in a systematic way (Livnat and Pippenger 2008). For example, in a choice between two alternative magnitudes (e.g. numbers, sucrose concentrations, or volumes), discrimination performance is expected to improve as the difference between the options increases (distance effect) and decline as

distance (the absolute difference between the two options) is kept constant but the average magnitude of the two options increases (magnitude effect, a consequence of the nonlinear compression of sensory information).

A well-established tradition uses honeybees (Apinae: Apini) and more recently bumblebees (Apinae: Bombini) as model organisms for studying foraging behavior and decision making (von Frisch 1927; Real 1981; Schmid-Hempel 1987; Schmid-Hempel and Schmid-Hempel 1987; Harder 1988; Waddington and Gottlieb 1990; Shafir et al. 2002, 2008; Heinrich 2004; Waldron et al. 2005; Cnaani et al. 2006; Gil 2010). However, despite investigations of the mechanisms of information processing in these insects (Waddington and Gottlieb 1990; Shafir 2000; Waddington 2001; Shafir et al. 2002, 2008; Waldron et al. 2005; Gil 2010), the relationship between information processing and choice profitability remains unclear. It has been demonstrated that bees form reward expectations (Gil 2010) and it has been suggested that the differences between the expectation and the actual perceived reward shape the development of economic flower preferences (Waldron et al. 2005; Wiegmann and Smith 2009). An important question that still needs to be addressed is how well bees track differences along reward dimensions while foraging under conditions similar to the natural situation, where there is uncertainty whether a flower contains any nectar.

In this study, I investigated the ability of the Common Eastern Bumblebee *Bombus impatiens* to discriminate between sucrose solutions with different sugar concentrations. Previous experiments have already shown that bumblebees are very sensitive to differences in sucrose concentration (Waddington 2001; Waldron et al. 2005; Cnaani et al. 2006; Wiegmann and Smith 2009). These studies suggest a nonlinear relationship between objective sucrose concentration (weight/weight percentage) and subjective evaluation (Waddington 2001) and indicate that foraging choices do not always conform to predictions based on net energy gain maximization (Schmid-Hempel 1987; Waldron et al. 2005; Cnaani et al. 2006). However, the precise functional relationship between discrimination performance and concentration has not yet been investigated.

A traditional psychophysical method for estimating discrimination performance is fitting a psychometric function to data from n-alternative forced choice tasks (*n*-AFC: Treutwein and Strasburger 1999). The psychometric function takes a measure of the intensities of the presented stimuli as argument and gives the discrimination performance, for example, the probability with which an observer judges one stimulus to be larger in magnitude from another stimulus. In previous two-alternative choice experiments with nectar-feeding bats (Chapter 1; Toelch and Winter 2007) the ratio of the linear difference of the

stimuli to the average stimulus value was proposed as the appropriate intensity measure, because it captures the expectations that discrimination performance should increase with the difference (distance effect) and decrease with the mean magnitude of the two options (magnitude effect).

The psychometric functions are typically assumed to have a sigmoidal shape and are modeled as the distribution functions of the normal, logistic, Weibull, or Gumbel distributions (Treutwein and Strasburger 1999; Kuss et al. 2005). Parameterization of the functions is preferably made so that the parameters have a meaningful biological interpretation, as is the case with the Weibull parameterization (Kuss et al. 2005; Fründ et al. 2011). The three parameters in the Weibull parameterization are the threshold, slope, and lapse rate. The threshold is the point on the curve that is halfway between the lower and the upper asymptote. In 2-AFC experiments, it usually corresponds to a discrimination performance around 75%. The slope of the function is measured at the threshold and has been proposed as a reliability measure of sensory performance (Strasburger 2001). Finally, the lapse rate is seen as a measure of the frequency of errors due to motivational problems and other factors of non-perceptual nature. The lapse rate is a measure that depends on the particular task given and I suggest that in animal studies, lapsing can also result from exploratory behavior (or from competition avoidance). Foraging animals face the exploration-exploitation dilemma and will not necessarily always make choices based on expected values. In psychometric analyses it is assumed that a forager has a constant lapse rate, that is, a constant probability to select an option not based on stimulus intensity. When a forager lapses during a specific choice in a 2-AFC experiment, its probability of selecting the correct option is at the chance level of 0.5 and equals the probability of selecting the incorrect option. Therefore, the lapse rate is calculated as one minus the upper asymptote of the psychometric curve (the estimated base rate of incorrect choices) multiplied by two.

To the best of my knowledge, a psychometric function for sugar concentration discrimination performance has so far only been fitted for one species, the nectar-feeding bat *Glossophaga commissarisi* (Chapter 1). The estimates for the lapse rate, threshold, and slope were 0.04, 0.50, and 3.41, respectively. In a recent dynamic modeling study of nectar extraction, the optimal sugar concentration for viscous dippers (animals that extract flower nectar by repeatedly dipping and retracting their tongues in the viscous liquid) was estimated at 52% w/w (Kim et al. 2011). However, although both bumblebees and bats are classified as viscous dippers (Kim et al. 2011), typical bat-pollinated plants have nectars with much lower sugar concentrations (13-18% w/w: Pyke and Waser 1981; Helversen and Reyer 1984) than

typical bee-pollinated plants (35% w/w: Pyke and Waser 1981). This difference cannot be explained by differences in nectar-drinking style as modeled by Kim et al. (2011). On the other hand, differences in discrimination performance between the two groups of pollinators might influence the evolution of nectar concentrations in the plants they pollinate. Since bumblebees live in an ecological environment with higher nectar sugar concentrations than flower bats, bumblebees may be expected to have a better developed ability for concentration discrimination. This is because of the magnitude effect. At the higher end of a perceptive scale, that is a higher sugar concentration, a higher sensitivity is required to discriminate between options that differ by a given distance in stimulus intensity. Here, I present the first psychometric analysis of sugar concentration discrimination performance in a nectar-feeding insect, based on two-alternative, free choice experiments with individually identifiable *B. impatiens* workers foraging on an array of computer-automated artificial flowers.

Methods

Bumblebees

I worked consecutively with two bumblebee colonies initially containing about 20-30 workers (Colony 1) and 40 workers (Colony 2) of *B. impatiens* (BioBest Canada Ltd, Leamington, ON, Canada). The experiments were carried out at the University of Toronto, Ontario, Canada. Nest boxes (29 × 21 × 14 cm) were connected by tunnels to a training cage (77 × 76 × 79 cm) where two artificial flowers (see below) provided nectar (sucrose aqueous solution, 20% w/w). After a training period of 6 days, the nest box was connected to one of the long walls of the test cage (293 × 245 × 219 cm) inside the same room. The test cage was equipped with six fluorescent lights providing a mixture of ultraviolet and white light. These lights were kept on a LD 12:12 schedule, while dimmed fluorescent white lights higher above the cage were kept on continuously. Commercial pollen was supplied as a food supplement directly to the colonies on a daily basis. I captured 75 foraging individuals and marked them with unique radiofrequency identification tags (RFID, PhenoSys, Germany). The tags were glued on the scuta of cold-anaesthetized bumblebees with cyanoacrylate glue (Instant KrazyGlue Gel Formula, Columbus, OH, USA). Bumblebees were then released in the test cage, where they could resume foraging.

Artificial Flowers

Visits to the artificial flowers or feeders (PhenoSys, Germany) were registered with an infrared sensor (Fig. 1). Transponder reading devices identified individuals carrying radiofrequency identification (RFID) tags. Each feeder was equipped with two solenoid pinch valves that controlled nectar delivery via two tubing systems (Fig. 1). Nectar rewards were delivered to a nectar bucket inside the feeder platform, a vertical hole with 5 mm diameter and 7 mm depth. The design of the nectar bucket was made after Ohashi et al. (2010) and included a plastic baffle to prevent bumblebees from getting nectar directly from the incoming tube (Fig. 1a). Nectar volume and concentration were controlled by two syringe pumps (PhenoSys, Germany) using two gas-tight Hamilton glass syringes (Series 1002, total volume 2.5 ml). After delivering a 5-µL reward, a feeder became unrewarding for 10 s, as an incentive for bumblebees to search for nectar elsewhere rather than collect multiple rewards at the same feeder. I assumed that bumblebees collected the full reward volume on every visit. If a bumblebee obtained a reward at a feeder and remained on it for longer than 10 s, it would need to leave the receptive field of the feeder's sensors in order to terminate the visitation event, before a further reward could be delivered. As bumblebees foraged simultaneously, the probability that a feeder would be unrewarding depended on the activity of the foragers, a situation that mimics natural foraging conditions. In order to make feeders more conspicuous and to promote learning, I adhered triangular "petals" made from colored electrical tape to the feeder platforms. I used red and white tapes for the two training feeders and blue and yellow tapes for the feeders in the main experiment. For the main experiment, I used a staggered 4 × 5 array of twenty computer-controlled feeders (Fig. 1b). Feeders were mounted on inverted flower pots, positioning the top of the landing platform approximately 10 cm above the floor. Feeders were spaced 40 cm apart. The whole array was positioned on the floor inside the test cage, about 50 cm from the two short walls and the long wall opposite the entrance point. The control computer, hardware interface, power supply units, and nectar reservoirs were all placed on a laboratory cart outside the back of the cage and connected to the feeders via signal cables and main nectar tubes. One pumping system supplied the blue-petaled feeders, the other the yellow. The two systems were filled with nectars with different concentrations. Thus, during a single experimental session, the concentration offered at each feeder was fixed and did not change. In order to prevent bacterial and fungal growth inside the tubing systems, they were rinsed with water and a 70%-ethanol solution every 3-4 days.

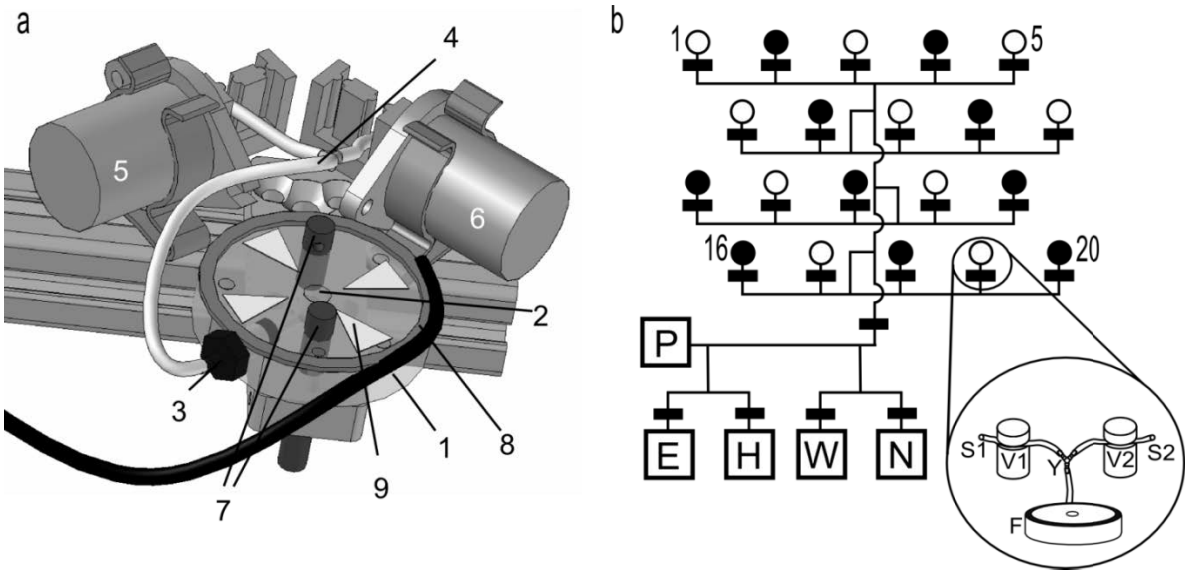

Figure 1. Artificial bumblebee flowers. **a** Schematic view of a single flower. Bumblebees land on an acrylic platform (*1*) and collect nectar from the nectar bucket (*2*). The bucket is filled through a horizontal hole connected via a thread-to-barb connector (*3*) to a nectar tube (*4*). Direct access to the nectar in the horizontal hole is prevented by a plastic baffle (not shown here, see Ohashi et al. (2010) for details). The tube receives nectar from either one of the two pumping systems, the tubes of which merge with (*4*). Nectar supplied from the two systems is directed to *4* by pinch valves (*5* and *6*). The delivery of nectar can be triggered when the infrared sensor's (*7*) light beam is interrupted. If the bumblebee carries a tag, its unique number is detected by an antenna (*8*) and sent to the transponder reading device (not shown). Flower nectar quality is indicated with color cues: triangular "petals" cut from electrical tape (*9*) and adhered to (*1*). **b** Pump and tubing system of the 20-feeder array**.** *Lines* represent the tubes, and *black rectangles* the pinch valves. Feeders are numbered *1-20*. *Boxes* represent the following liquid reservoirs: ethanol (*E*), water (*H*), waste (*W*), nectar (*N*), stepping-motor syringe pump (*P*), as described in Winter and Stich (2005). Length of tubes not drawn to scale. Two identical tubing systems were connected to the feeders. The merging point of the tubing systems is illustrated in the inset: magnetic pinch valves for the first (*V1*) and second (V2) tubing systems with their corresponding tubes (*S1* and *S2*), a Y connector (*Y*) and feeder platform (*F*). Feeders shown in black had blue "petals" and only received nectar from S1, and feeders shown in white had yellow "petals" and only received nectar from *S2*. The two pumping systems were filled with different sugar concentrations on different days. See "Methods" for details

Experimental Procedure

Bumblebees were first trained on the two feeders inside the training cage for 6 days, and then their nest box was connected to the experimental cage. The tunnel that connected the colony to the cage was kept closed during the "dark" phase and opened within 1-2 hours after

the start of the "light" phase. On the first experimental day for each colony, the entrance to the cage was smeared with honey, as an incentive for bumblebees to explore the cage. On the following days, bumblebees spontaneously left their nest box as soon as the connecting tunnel was opened. A foraging session began with the opening of the connecting tunnel and ended 12 h later, when reward delivery at the feeder array was automatically stopped. Most bumblebees would then spontaneously return to their nest box. The remaining individuals were netted and placed in the nest box. All feeder visits during a foraging session were recorded.

I chose concentrations from the natural range of floral nectars (Pyke and Waser 1981), ranging from 15 to 50% sucrose/water weight/weight (or 464-1796 mmol L^{-1}, Bolten et al. 1979). For each of the two colonies I conducted a series of two-alternative free-choice tests, with 10 feeders per option (Table 1). Every concentration pair was presented twice on consecutive days, with the positions of the two concentrations exchanged as a control for positional and color biases (Fig. 1b). This resulted in reversal test conditions for the bumblebees on nearly every day (Table 1). All experiments were performed with PhenoSys (Germany) experimental control software.

Data Analysis

Recorded data comprised the time-stamped visitation events of marked and identified and of unmarked bumblebees. In order to focus on the plateau performance of bees that had become familiar with the choices being offered, after the initial sampling and exploration phase, I excluded the first 800 visits from the analysis of marked bumblebee data. Visual inspection of the daily learning curves confirmed that no substantial changes in feeder preference occurred after the 800-visit cut-off point. A total of 34 marked bumblebees made at least 50 visits on at least 1 day and 13 marked bumblebees made at least 800 visits on at least 1 day. Out of these individuals, three bumblebees retained their transponders for a sufficient number of days and made a sufficient number of visits to permit individual-based psychometric analyses for these three animals. The three individuals came from the second colony. Otherwise, I analyzed unmarked bumblebee data collectively. I estimated the number of visits per bumblebee by taking the recorded mean daily visits by the 34 marked bumblebees that made at least 50 visits on at least 1 day. I then estimated the number of foraging individuals by dividing the total number of unmarked visits by the estimate for the number of visits per bumblebee. For the asymptotic performance of the unmarked

bumblebees, I assumed the same cut-off point of 800 visits per bee and approximated it by excluding the first *m* visits, where *m* was 800 multiplied by the estimated number of unmarked individuals. For each marked bumblebee, and for the unmarked bumblebees from each colony, I calculated the *relative intensities* (treatment) and the *discrimination performances* (response) for each experimental day. The *relative intensity* was calculated as the absolute difference between the two sucrose concentrations expressed in percentage weight/weight, divided by the mean concentration. Here, I adopt this measure on theoretical grounds (Chapter 1; Toelch and Winter 2007) without explicitly testing the separate contributions of the distance and magnitude effects. The response was calculated as the number of visits to higher concentration feeders divided by the total number of visits. I calculated separate responses for each day; for further analyses, I combined the daily responses as the weighted average over the two presentations of the same condition, using number of registered visits as weights. This step was intended to control for positional or color biases. Statistical analysis was carried out using R 2.10.1 (R Development Core Team 2009).

Psychometric Analysis

I performed psychometric analyses on the response data from each animal and each colony (unmarked bumblebees) and fitted Weibull psychometric functions using the algorithm proposed by Kuss et al. (2005) with *relative intensity* as independent and *discrimination performance* as dependent variables (Chapter 1; Toelch and Winter 2007). This Bayesian approach yields estimates for the threshold, slope, and lapse rate of the psychometric function, as well as confidence intervals for these parameters, using Markov Chain Monte Carlo (MCMC) sampling. For the threshold, I chose a normally distributed prior with a mean of 1 and a standard deviation of 0.5, and for the slope, a normal prior with a mean of 2 and a standard deviation of 1. In human experiments, the lapse rate is usually in the range 0.01-0.10, but instead of restricting the prior to this range, I selected as prior the beta distribution (2;20), in order to allow for higher lapse rates due to exploratory behavior. I performed 5,000 MCMC sampling runs with a leapfrog step size of 100 to obtain the mean and 95% confidence intervals for the threshold, slope, and lapse rate.

Table 1 Discrimination performance (response) for different sucrose concentrations in *B. impatiens* workers from two different colonies

Day	Blue[a]	Yellow[a]	Average[a]	Intensity[b]	*N* bees[c]	*N* visits × 1,000[d]	Response[e]
Colony 1							
1	30	15	22.5	0.67	0 (0)	- (-)	- (-)
2	15	30	22.5	0.67	1 (5)	0.59 (0.90)	0.96 (0.98)
3	45	30	37.5	0.40	1 (4)	1.04 (0.76)	0.87 (0.94)
4	30	45	37.5	0.40	0 (6)	- (0.77)	- (0.95)
5	30	20	25.0	0.40	0 (7)	- (0.94)	- (0.87)
6	20	30	25.0	0.40	1 (9)	0.02 (1.52)	0.92 (0.88)
7	45	50	47.5	0.11	1 (8)	1.90 (1.71)	0.71 (0.7)
8	50	45	47.5	0.11	0 (9)	- (1.82)	- (0.62)
9	35	45	40.0	0.25	0 (6)	- (0.86)	- (0.79)
10	45	35	40.0	0.25	1 (9)	1.20 (1.85)	0.83 (0.8)
11	30	32	31.0	0.06	1 (7)	0.11 (1.62)	0.45 (0.48)
12	32	30	31.0	0.06	1 (9)	0.44 (1.50)	0.49 (0.46)
13	40	20	30.0	0.67	2 (13)	3.01 (2.39)	0.89 (0.89)
14	20	40	30.0	0.67	2 (12)	4.45 (1.98)	0.92 (0.9)
15	35	39	37.0	0.11	2 (14)	1.49 (2.56)	0.83 (0.67)
16	39	35	37.0	0.11	1 (17)	0.47 (3.12)	0.56 (0.63)
Colony 2							
1	50	45	47.5	0.11	5 (20)	3.35 (5.01)	0.52 (0.57)
2	45	50	47.5	0.11	2 (10)	0.06 (2.40)	0.52 (0.57)
3	45	30	37.5	0.40	0 (11)	- (2.73)	- (0.85)
4	30	45	37.5	0.40	4 (9)	2.71 (2.13)	0.96 (0.92)
5	25	20	22.5	0.22	3 (9)	1.20 (2.43)	0.54 (0.65)
6	20	25	22.5	0.22	4 (11)	5.51 (2.40)	0.6 (0.62)
7	30	15	22.5	0.67	4 (12)	5.64 (2.94)	0.87 (0.84)
8	15	30	22.5	0.67	5 (12)	5.31 (3.47)	0.88 (0.79)
9	34	25	29.5	0.31	3 (12)	2.65 (2.89)	0.93 (0.91)
10	25	34	29.5	0.31	3 (16)	2.92 (3.53)	0.83 (0.66)
11	27	21	24.0	0.25	4 (16)	4.36 (3.50)	0.58 (0.56)
12	21	27	24.0	0.25	3 (14)	3.16 (3.08)	0.72 (0.68)

Bees were presented with 20 artificial flowers with blue ($N = 10$) and yellow petals ($N = 10$) and the relative preference for the feeders with the sweeter nectar was calculated for bumblebees that made at least 800 visits

[a] Sucrose solution concentrations are given in % weight/weight

[b] Relative intensity is calculated as the difference between the two concentrations (blue and yellow) divided by the average of the concentrations

[c] Numbers without parentheses give the number of marked bumblebees that made at least 800 visits. Numbers in parentheses give the number of unmarked bumblebees, estimated by dividing the total number of recorded unidentified visits for that day by the average number of visits per day for unmarked bumblebees over the whole experiment of the respective colony

[d] Numbers without parentheses give the total number of visits (in thousands) made by marked bumblebees, excluding the first 800 visits per individual. Numbers in parentheses give the total number of visits (in thousands) made by unmarked bumblebees, excluding the first *m* visits, where *m* is 800 × estimated number of unmarked individuals

[e] Numbers without parentheses give the weighted average response of marked bumblebees using the individual number of visits as weights. Numbers inside parentheses give the response of unmarked bumblebees. The marked and unmarked bumblebee responses were positively correlated in Colony 1 (Spearman Rank correlation = 0.92, $S = 18$, $p < 0.001$, $N = 11$ days) and in Colony 2 (Spearman Rank correlation = 0.91, $S = 20.55$, $p < 0.001$, $N = 11$ days)

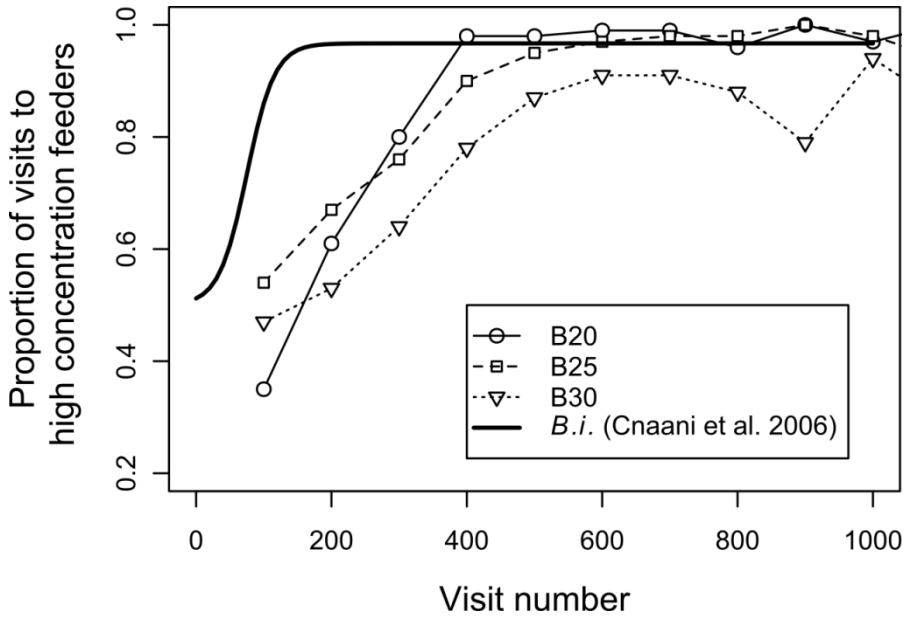

Figure 2. Learning curves for *B. impatiens* in sucrose discrimination tasks. *Open symbols* give the proportion of visits to the higher concentration feeders calculated over bins of 100 visits. For each of the three focus individuals from this study, the steepest learning curves from the complete data sets were selected. For B20, B25, and B30, data were taken from the first 1000 visits on days 4, 8, and 7, respectively. The *thick line* is calculated from the learning curve parameters estimated in a previous study with *B. impatiens* (Cnaani et al. 2006: Table 2, "Concentration 30"). The concentrations used in that study were 13% and 40% (weight/weight)

Results

On average, each of the 34 marked bumblebees made 1,076 ± 642 visits bee^{-1} day^{-1} (mean ± SD, excluding individuals which made fewer than 50 visits, $N = 27$ days) and the

total of unmarked bumblebees made 10,754 ± 4, 065 visits day^{-1} (mean ± SD, N = 27 days). Even after the first 800 visits, marked bumblebees usually continued to visit almost all of the 20 available feeders (mean ± SD = 18.5 ± 1.63 feeders, N = 15 bumblebees) thus visiting both concentration types. However, they seldom distributed their visits evenly among the feeders. Even at the highest relative intensities, bumblebees made at least 400-600 visits before reaching asymptotic performance in their choice behavior (Fig. 2). They showed no discrimination between concentrations at low relative intensities and good discrimination at high relative intensities. This led to psychometric functions that are nonlinear for the variables I have chosen (Fig. 3).

In Colony 1 the proportion of non-rewarded visits (visits within the 10-s refill delay) at feeders with lower concentration (mean ± SD = 0.47 ± 0.10, N = 15 days) was the same as at feeders with higher concentration (mean ± SD = 0.48 ± 0.06, N = 15 days; paired t test: $t(14) = -0.64$, $p = 0.53$). In Colony 2 the feeders with lower concentrations had a lower frequency of non- rewarded visits (mean ± SD = 0.54 ± 0.03, N = 12 days) than feeders with higher concentrations (mean ± SD = 0.58 ± 0.03, N = 12 days; paired t test: $t(11) = -5.39$, $p < 0.001$), but the difference was small.

As seen in Table 2, the psychometric function thresholds estimated from individually analyzed marked bumblebees (mean ± SD = 0.25 ± 0.01, N = 3 bumblebees) were similar to the values obtained from pooling miscellaneous marked bumblebees (0.24) and similar to the values from all unmarked bumblebees (0.22). The individually estimated lapse rates (mean ± SD = 0.23 ± 0.11, N = 3 bumblebees) were also similar to the estimates obtained from pooling miscellaneous marked bumblebees (0.18) and all unmarked bumblebees (0.25). Finally, the psychometric function slopes varied strongly from individual to individual (mean ± SD = 8.22 ± 3.80, N = 3 bumblebees), and the corresponding estimates for miscellaneous marked individuals and for all unmarked individuals were lower, at 3.29 and 3.12, respectively (Table 2).

Discussion

The bumblebees could choose between two types of sugar solutions that differed on different experimental days in their relative intensity to each other. Depending on relative intensity of difference between options, *B. impatiens* workers were either indifferent to differences in sucrose concentration or made more visits to the feeders with the higher

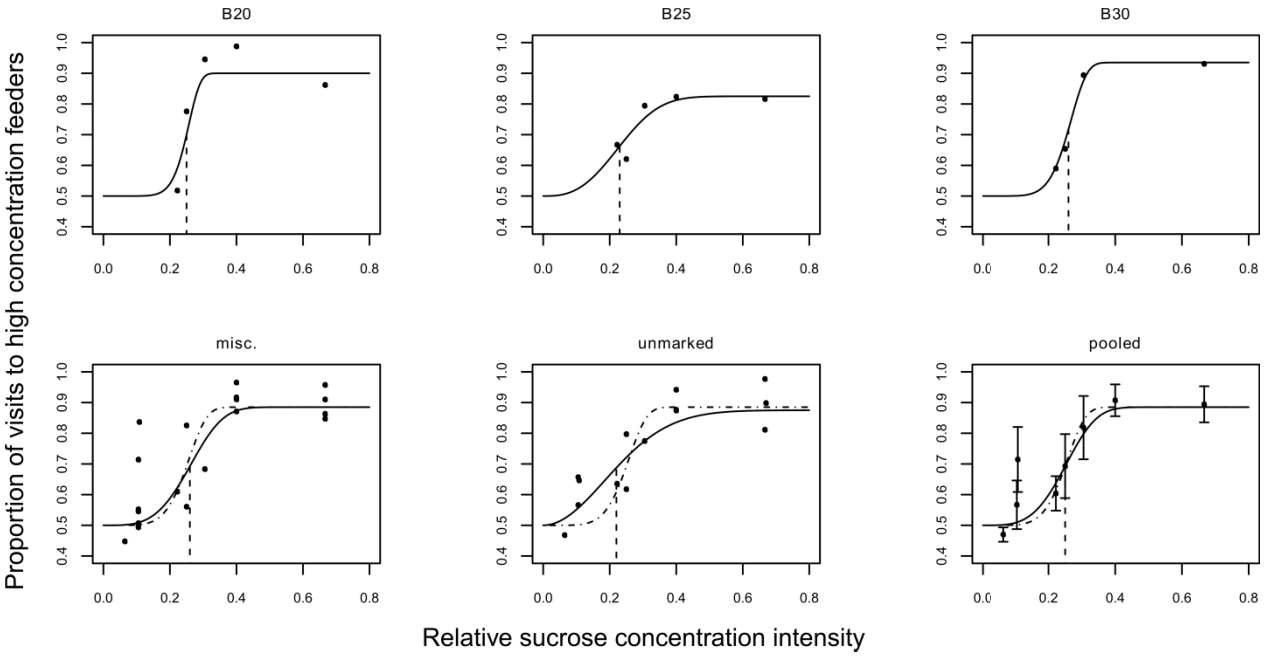

Figure 3. Psychometric curves for sucrose concentration discrimination. Sucrose concentration intensities are given on the abscissa and are calculated as the absolute value of the difference divided by the mean of two concentrations (see "Methods"). *Black circles* represent weighted average responses (proportion of visits to the higher sucrose concentration) over two presentations of the same pair of sucrose concentrations (Table 1), using number of visits as weights. The *continuous curves* represent the respective psychometric functions and the *dashed vertical lines* indicate the psychometric function thresholds. The top three *panels* from left to right give data from three individually marked bumblebees. The bottom left *panel* gives the weighted average responses of marked bumblebees from both colonies that satisfied the minimum 800 visits per day criterion, but were not detected on a sufficient number of days for individual psychometric analysis. (Most of these data points are for single days only, rather than average values over 2 days.) The bottom middle panel gives the weighted average responses of all unmarked bumblebees from both colonies and the bottom right panel gives the average responses (*circles*) and standard deviations (*whiskers*) calculated from pooling all data together (B20, B25, B30, miscellaneous, unmarked). The dashed curves in the bottom panels represent the psychometric function with parameters (lapse rate, threshold, and slope) averaged over the parameters of the three individually marked bumblebees

concentration. Their discrimination performance can be described by the psychometric function presented in this study (Fig. 3). In general, the predicted relative visitation rate to the sweeter option of two concentrations (from the range 15-50% w/w) with relative intensity x can be calculated with the following equation:

$$\Psi(x,m,s,\pi_l) = \frac{1}{2}\left[\pi_l + (1-\pi_l)\left[2 - \exp\left(-\exp\left(\frac{2sm}{\ln(2)}(\ln(x)-\ln(m)) + \ln(\ln(2))\right)\right)\right]\right] \quad (1)$$

where m is the threshold, s is the slope at the threshold, and π_l is the lapse rate (from equations (1) and (11) in Kuss et al. 2005). For instance, the psychometric function predicts that for intensities higher than the threshold (x > 0.25, Table 2.), the options with the more concentrated nectars will receive at least 70% of all visits. Because of the somewhat high estimated lapse rates (Fig. 3, Table 2), the psychometric function likely underestimates the perceptual capacity for sugar discrimination in bumblebees. Caution should also be taken when using concentrations higher than 50% w/w, as viscosity and extraction costs are known to increase with concentration (Harder 1986; Kim et al. 2011) and may invalidate predictions based on the psychometric function. Whether that is the case could be tested by disassociating viscosity from sweetness using the inert polymer Tylose (Josens and Farina 2001; Borrell 2006; Köhler et al. 2010).

When comparing the individually calculated psychometric functions with functions fitted on pooled data from unmarked or miscellaneous marked bumblebees (Fig. 3, Table 2), the different data sets yield similar estimates for the threshold (all in the range 0.22-0.26) and

Table 2 Psychometric function parameters for discrimination of sucrose solution concentrations in *B. impatiens* workers

Bumblebee	Lapse Rate[a]	Threshold[a]	Slope[a]	*N* (days)
B20	0.19 | 0.20 | 0.21	0.244 | 0.247 | 0.25	10.80 | 11.67 | 12.59	7
B25	0.33 | 0.35 | 0.38	0.22 | 0.23 | 0.24	3.19 | 4.15 | 5.08	9
B30	0.12 | 0.13 | 0.14	0.256 | 0.26 | 0.263	8.05 | 8.82 | 9.62	7
misc.[b]	0.17 | 0.18 | 0.19	0.23 | 0.24 | 0.26	2.68 | 3.29 | 4.35	24
unmarked[c]	0.24 | 0.25 | 0.26	0.21 | 0.22 | 0.23	2.95 | 3.12 | 3.29	27
pooled[d]	0.22 | 0.23 | 0.23	0.248 | 0.251 | 0.253	4.80 | 5.30 | 5.80	27

[a] Parameters estimated with a Bayesian Markov Chain Monte Carlo (MCMC) sampling method (Kuss et al. 2005). Values in the middle are average estimates and the values to the left and right are the 95% confidence interval limits

[b] Analysis based on pooled data from miscellaneous marked bumblebees that made sufficient number of visits on some days, but were not detected over a sufficient number of days for individual psychometric analysis (N = 10 bumblebees)

[c] Analysis based on data from all unmarked bumblebees

[d] Analysis based on pooled data from all bumblebees (B20, B25, B30, misc., and unmarked)

are consistent with respect to the lapse rate (all in the range 0.18-0.25). As shown in the results and in Fig. 4, the slope is underestimated when pooled data from unmarked or miscellaneous marked bumblebees are analyzed instead of separately analyzing individual data. I conclude from this that if researchers are primarily interested in estimating the threshold rather than the slope, then similar psychometric studies (e.g. on nectar volume, or probability of reward) can be conducted without the individual transponder tracking used in this study.

Gustatory perception of sucrose concentration depends on chemoreceptors on bees' glossae (Whitehead and Larsen 1976), and evaluation of this information is probably immediate. Yet bumblebees needed several hundred visits to reach asymptotic performance in their choice behavior (Fig. 2). The lower learning rates in comparison with the rates reported by Cnaani et al. (2006) may possibly reflect the difficulty of performing a spatial reversal task in my experiments. I interchanged the positions of higher and lower quality feeders in the experimental array daily. Impeded learning could also be explained by differences in salience of the sensory cues (visual vs. olfactory) or by a possible confounding effect of the 10-s delay rule (see Methods), which led to ca. 50% unrewarded visits.

The psychometric function predicts that bumblebee workers will be indifferent to sugar concentration differences below a relative intensity value of about 0.1. However, strong preferences for one feeder type over the other were detected in some marked bees even below this value (Table 1, Colony 1, days 7 and 15; see also Fig. 3, bottom left panel, points at 0.11 relative intensity). This discrimination performance may have been facilitated by a carryover effect from the previous day providing a learning phase with 2-day duration. On experimental days 7 and 15, in deviation from regular routine, there was no reversal with respect to the previous days, that is, the higher concentrations were in the same colored feeders for two consecutive nights (Table 1). It appears that in the absence of strong sugar concentration differences, some bumblebees did not update the remembered value of the lower concentration type as fast as others.

It has been hypothesized that the difference between reward expectation and actual perceived reward drives the choice for more profitable food options in bees (Waldron et al. 2005; Wiegmann and Smith 2009). There is some field evidence that bumblebees employ a win-stay, lose-shift strategy: when they consecutively experienced low reward volumes (estimated by measuring flower handling time as proxy) at one flower species, they were more likely to switch to another species (Chittka et al. 1997; but see Bar-Shai et al. 2011).

In addition to the difference between the two sucrose concentrations, the bumblebees in my experiment could also experience unrealized reward expectations when making a non-rewarded visit at each feeder type. One way to demonstrate a negative incentive contrast of this kind is to show that after experiencing two unrewarded visits at high concentration feeders (e.g. blue), bumblebees are more likely to sample a low concentration feeder (e.g.

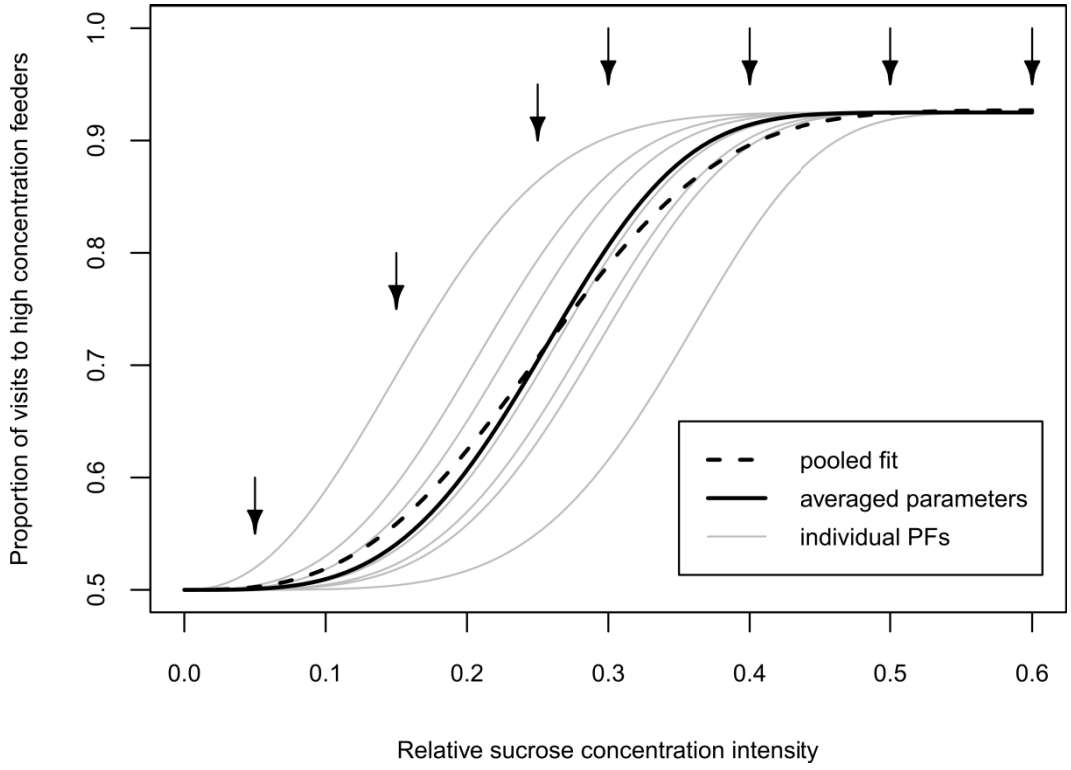

Figure 4. Data pooling can cause underestimation of the psychometric function slope. The figure illustrates with a theoretical example how the averaging of individual data changes psychometric function parameters. I start with 7 "individuals" represented by psychometric functions (PFs, *gray lines*) with different thresholds (mean ± SD: 0.25 ± 0.057), but equal lapse rates (0.15) and slopes (5). From the individual curves, I calculate the predicted discrimination performance values at relative intensities 0.05, 0.15, 0.25, 0.3, 0.4, 0.5, and 0.6 (*arrows*). I then average the predicted discrimination performances across animals using 200 visits per animal for each intensity value (N = 200 visits × 7 animals = 1400 visits per relative intensity value) and apply the algorithm for psychometric function fitting by Kuss et al. (2005). I use a flat prior for the slope, in order to exclude potential confounding effects of the prior and select all remaining parameters as described in the "Methods" section. The resulting psychometric curve (*dashed line*) has a slope (± 95% CI) of 4.07 ± 0.67, significantly lower than the actual value of 5 that was identical for all individuals in the initial theoretical functions ($p <$ 0.05). The estimates for the lapse rate (0.15 ± 0.02) and threshold (0.25 ± 0.01) do not differ from the average parameters. For comparison, the psychometric curve with parameters averaged across animals is also shown (*continuous black line*)

yellow) than after experiencing a reward followed by a non-rewarded visit at blue feeders (Prediction 1). (Hereafter, I refer to the high concentration feeders as blue and low concentration feeders as yellow for ease of explanation). Similarly, if the remembered value of a feeder is downgraded after a non-rewarded visit, then bumblebees should be more likely to sample a yellow feeder after making two unrewarded visits at blue feeders than after making two rewarded visits at blue feeders (Prediction 2). In order to test these predictions, I looked at the first 800 visits marked bumblebees made on days with relative intensity of 0.67 (the condition with the highest number of detected marked bumblebees). I excluded animals if they did not develop a preference above 90% for blue feeders and performed paired t tests with probability to shift from blue to yellow as the dependent variable and the last two reward experiences (two rewards, or one reward followed by no reward, or two unrewarded visits) as the independent variable. My results failed to support Prediction 1 (paired $t(6) = -1.989$, $p = 0.09$, $N = 7$ bumblebees) and Prediction 2 (paired $t(6) = -2.454$, $p = 0.0495$, $N = 7$ bumblebees). In both cases, the differences were in the opposite direction of the predicted, that is, bumblebees were more likely to shift to yellow after experiencing two rewards at blue feeders than after experiencing two non-rewarded visits at blue feeders. My interpretation of these results is that bumblebees do not update the expected value of color marked feeders when experiencing non-rewarded visits.

Despite the uncertainty and frequent changes in feeder quality, the psychometric function that describes the discrimination performance of *B. impatiens* workers is finely tuned, with a lower threshold (0.25) and a steeper slope (5.3) than the mean threshold (0.50) and slope (3.3) of *G. commissarisi* bats measured in a similar two-alternative free choice task (Chapter 1). In other words, bumblebees seem to be better at discriminating small differences between sugar concentrations than nectar-feeding bats. As described in the introduction, bumblebee-pollinated plants have on average sweeter nectars than bat-pollinated plants. Here, I show that the groups also differ in their psychometric functions of sweetness perception. This raises the question how the evolution of plant nectar traits and pollinator information-processing mechanisms might be related.

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CHAPTER 3: Weber's law, the magnitude effect, and discrimination of sugar concentrations in nectar-feeding animals

Abstract. Weber's law quantifies the perception of difference between stimuli. For instance, it can explain why we are less likely to detect the removal of three nuts from a bowl if the bowl is full than if it is nearly empty. This is an example of the magnitude effect – the phenomenon that the subjective perception of a linear difference between a pair of stimuli progressively diminishes when the average magnitude of the stimuli increases. Although discrimination performances of both human and animal subjects in various sensory modalities exhibit the magnitude effect, results sometimes systematically deviate from the quantitative predictions based on Weber's law. An attempt to reformulate the law to better fit data from acoustic discrimination tasks has been dubbed the 'near-miss to Weber's law'. Here, I tested the gustatory discrimination performance of captive nectar-feeding bats (*Glossophaga soricina*), in order to investigate whether the original version of Weber's law accurately predicts choice behavior in a two-alternative forced choice task. As expected, bats either preferred the sweeter of the two options or showed no preference. In 4 out of 6 bats the near-miss to Weber's law provided a better fit and Weber's law underestimated the magnitude effect. In order to test the generality of this observation in nectar-feeders, I reviewed previously published data on bats, hummingbirds, honeybees, and bumblebees. In all groups of nectar-feeding animals the near miss to Weber's law provided better fits than Weber's law. Furthermore, whereas the magnitude effect was stronger than predicted by Weber's law in vertebrates, it was weaker than predicted in insects. Thus nectar-feeding vertebrates and insects differed in how their choice behavior changed as sugar concentration increased. I discuss the ecological and evolutionary implications of the observed patterns of sugar concentration discrimination.

Introduction

The capacity of decision-makers to make choices that maximize profitability crucially depends on their ability to sense and evaluate differences among alternative choice options (Kacelnik and Brito e Abreu 1998; Livnat and Pippenger 2008; Shafir et al. 2008). When sequential sampling of multiple options takes place, decision-makers rely on sensation (converting a physical stimulus into a neuronal firing pattern), memory (maintaining a representation of a physical stimulus over a period of time) and decision making (comparing representations from different sources and performing a motor task based on the results of this comparison). Taken together these three processes constitute what I refer to as "information processing".

In information processing comparison of stimuli is assumed to occur on cognitive representations of physical scales (Fechner 1860; Thurstone 1927; Stevens 1961). As direct observations and measurements of subjective sensations are not possible, the relationships between the physical and the psychological scales are studied by measuring behavioral output or neuronal activity. A well-known and highly discussed psychophysical invariant is Weber's law (Fechner 1860; Stevens 1961; Perez and Waddington 1996; Kacelnik and Brito e Abreu 1998), which states that judgments of stimulus change are at a constant proportion of the stimulus magnitude. The law can be expressed with the Weber fraction

$$\Delta(a)/a = c \qquad (1)$$

where a is the magnitude (e.g. the length of a line measured in mm or the concentration of a sugar solution measured in % weight/weight, etc.) of a physical stimulus (standard stimulus), $\Delta(a)$ is the difference between the magnitude a and the magnitude of a second (referent) stimulus that is *just noticeably different* (*jnd*) for an observer, and c is a constant that depends on the observer and the sensory modality. Modern psychophysics considers not just the *jnd*, but a family of so-called sensitivity functions ξ. For a fixed magnitude a and a discrimination probability π a sensitivity function $\xi_\pi(a)$ gives the magnitude of a stimulus that is judged greater than a with probability π. With sensitivity functions a differential threshold at any chosen probability π can be defined as $\Delta_\pi(a) := \xi_\pi(a) - a$. Weber's law can thus be reformulated as $\Delta_\pi(a)/a = c$ for any π. In experimental settings the probabilities $p(x,a)$ (*discrimination performances*) that a stimulus with a magnitude x is judged greater than a stimulus with magnitude a are measured usually in two-alternative forced choice (2AFC) or similar tasks and form the so-called psychometric function $P_a(x) := p(x,a)$. The sensitivity function is the inverse of the psychometric function $\xi_\pi(a) := P_a^{-1}(\pi)$. If Weber's law holds and

if we pick two pairs of stimuli with equal Weber fractions $\frac{x-a}{a} = \frac{y-b}{b}$ then $p(x,a) = p(y,b) = \pi$, with $x = \xi_\pi(a)$ and y = $\xi_\pi(b)$. I now define the *relative intensity* of two stimuli with magnitudes x and a with $x > a$ as

$$i(x,a) := \frac{x-a}{\frac{x+a}{2}}. \qquad (2)$$

Note that if Weber's law holds, then $i(x,a) = i(y,b)$ also implies that $p(x,a) = p(y,b)$. In other words if two concentration pairs have the same relative intensity, then an observer is expected to select the higher concentration stimulus with the same probability in each pair of options.

In a choice between two magnitudes discrimination performance usually improves as the difference between the options increases (distance effect) and it usually declines as distance (the absolute difference between the two options) is kept constant but the average magnitude of the two options increases (magnitude effect). Relative intensity and the Weber fraction are both ratios that combine these two effects. Mathematicians have demonstrated that as long as discrimination probabilities are determined by differences in psychophysical scale values, the choice of measurement of the physical scale is immaterial (Iverson 2006). The advantage of relative intensity over the Weber fraction is that it can be used also in situations where the dichotomy between standard and referent option does not apply, i.e. there is symmetry in the presentation of the two stimuli, so that they both can be seen as a standard. In theory, this allows the construction of a single function relating any two magnitudes to discrimination performance rather than a family of different functions for the different standards used. Thus, a psychometric function can also be defined with respect to relative intensity: $\psi(i) = \psi(i(x,a)) := P_a(x)$.

Psychometric functions are usually assumed to be sigmoid functions such as the distribution functions of the normal, logistic, Weibull, and Gumbel distributions (Treutwein and Strasburger 1999; Wichmann and Hill 2001; Kuss et al. 2005; Augustin 2008). The Weibull function has the advantage that it can be parameterized in terms of biologically meaningful parameters, the threshold, slope, and lapse rate (Treutwein and Strasburger 1999; Wichmann and Hill 2001; Kuss et al. 2005; Augustin 2008; Fründ et al. 2011). The point on the curve halfway between the lower and upper asymptote (corresponding to a discrimination performance of ca. 75%) is referred to as the threshold. The slope of the function at the threshold is interpreted as a reliability measure of sensory performance (Treutwein and Strasburger 1999). An important distinction needs to be made at this point between discrimination performance and the capacity to discriminate. At stimuli with very high

relative intensity corresponding to the upper asymptote of a psychometric curve it is presumed that an observer's capacity to discriminate has reached 100%. The actual measured discrimination performance is usually lower than expected, because of lapsing, that is, making a decision that is not based on relative intensity but may constitute an error due to motivational problems or other factors of non-perceptual nature. The lapse rate depends on the particular task given and I suggest that in animal studies lapsing can also result from exploratory behavior. Foraging animals face the exploration-exploitation dilemma and will not necessarily always make choices based on expected values. In psychometric analyses it is assumed that an observer has a constant lapse rate, that is, a constant probability to select an option not based on relative intensity but using an alternative rule. The lapse rate is calculated as one minus the upper asymptote of the psychometric curve multiplied by two.

Near-Miss to Weber's Law

Empirical tests of Weber's law in the fields of acoustical (McGill and Goldberg 1968; Doble et al. 2003) and visual (Augustin and Roscher 2008) perception have revealed that for very high stimulus magnitudes observers perform better than predicted. (Fechner (1860) pointed out that the Weber fraction remains constant only for a limited range of stimulus magnitudes.) Discrimination performances in these studies are better fitted by an expression that allows sensitivity to grow as a power function of stimulus magnitude (McGill and Goldberg 1968; Doble et al. 2003; Iverson 2006; Augustin and Roscher 2008; Augustin 2008, 2009):

$$\xi_\pi(a) = K(\pi)\, a^{\beta(\pi)} \tag{3}$$

where $K(\pi)$ and $\beta(\pi)$ are real valued parameters that may depend on the value of π. If the value of $\beta(\pi)$ is one then Weber's law is satisfied. Equation 3 has been demonstrated to hold over a wide range of magnitudes and because the exponent β is typically estimated around 0.9, equation 3 is referred to as the near-miss to Weber's law (Doble et al. 2003; Augustin and Roscher 2008; Augustin 2008, 2009).

Here I consider a slightly different formulation of the near-miss to Weber's Law and define the *near-miss relative intensity* of two stimuli with magnitudes x and a with $x > a$ as

$$\rho(x,a) := \frac{x-a}{\left(\frac{x+a}{2}\right)^{\beta}}. \tag{4}$$

The parameter β determines how strong the magnitude effect is with respect to the distance effect and if it equals 1, then near-miss relative intensity reduces to relative intensity. Thus, I consider Weber's law to be satisfied when the parameter β is estimated to be one and invoke the near-miss to Weber's law when β significantly differs from one.

Knowledge of gustatory information processing (Perez and Waddington 1996; Norwich 1984; Papini and Pellegrini 2006) is important for understanding the formation of economical food preferences (Waldron et al. 2005; Toelch and Winter 2007; Chapters 1–2) and may have implications for our understanding of the co-evolution of floral nectar and pollinator discrimination. Diverse groups of nectar-feeding animals such as bees (Chapter 2; von Frisch 1927; Cnaani et al. 2006), birds (Hainsworth and Wolf 1972; Roberts 1996; Nicolson and Fleming 2003) and bats (Chapter 1; Roces et al. 1993; Rodríguez-Peña et al. 2007) show a general pattern of preference for sweeter sugar solutions and more precise discrimination at low concentrations, a pattern that is qualitatively consistent with Weber's law. In glossophagine bats (Chapter 1) the discrimination probability in a choice between nectars with different sugar concentrations can be reasonably fitted against relative intensity. Although the results were consistent with Weber's law, the law was not rigorously tested in these studies. Here, I present a series of 2AFC tests with nectar-feeding bats (*Glossophaga soricina*) designed to test directly whether concentration pairs with the same relative intensity result in equal discrimination probabilities (as predicted by Weber's law). I also tested whether near-miss relative intensity is a better predictor of discrimination performance than relative intensity. I used the method of constant stimuli with a standard feeder giving rewards with 20% weight/weight sugar concentration and a test (referent) feeder, whose concentration was systematically varied. This allowed me to construct individual psychometric functions for the discrimination of sugar concentration. Finally, I reviewed previously published data on bats, hummingbirds, honeybees, and bumblebees in order to test whether Weber's law or the near-miss to Weber's law provide a better fit to sugar concentration discrimination performance.

Methods

Subjects

Experiments were carried out with five female and one male Pallas's long-tongued bats (*Glossophaga soricina*) from the same colony at Bielefeld University. The climatic

conditions in the housing room (ca. 2.2 × 3.4 × 3.7 m) were ca. 25 °C and ca. 60% humidity. The colony received ad libitum 20% honey water, honey water mixed with supplementary nutrients: Nektar Plus (Nekton®, Günter Enderle, Pforzheim, Germany) and Alete2Folgemilch (Nestle), Multi-Mulgat® (BioWeyxin, Veyx-Pharma GmbH, Schwarzenborn, Germany) and bee-collected flower pollen. Once a month they were also provided with live flies (*Musca domestica*). The six experimental individuals were adult, older than one year of age. Bats were marked with unique Radio Frequency Identification tags (RFID: 12 × 2.1 mm, 124 kHz, Sokymat) using self-made silicon collars (total collar and RFID weight = 0.20 g, less than 2.5% of the body mass of the smallest bat). After the experiment, the animals were returned to the colony. Light conditions during the experiments were 12:12 LD and all experiments were conducted during the dark phase. Treatment of the experimental animals complied with the national laws on animal care and experimentation, under license of Veterinäramt Bielefeld.

Cage and feeder system

During the experiments each bat was placed individually inside one of three adjacent cages (0.7 × 2.2 × 1.5 m, Winter and Stich (2005)), inside a 3.4 × 3.8 × 3.7 m room. Each cage contained two computer-controlled feeders on the back wall and a hanging roost. Visits to the feeders were automatically detected by infrared sensors. Upon detecting a visitor, feeders delivered a fixed amount of 20 µL sugar water (hereafter 'nectar') as a reward that the bats removed by licking while briefly hovering in front of a feeder. Nectar reward delivery was controlled by two syringe pumps using two gas-tight Hamilton glass syringes (Series 1025). Feeders were connected to the pumps via two identical systems of pinch valves and tubes (Fig. 1). Access to each feeder could be blocked automatically by moving a swivel arm with a plastic guard in front of the feeder opening. Details of the experimental apparatus are given in Winter and Stich (2005).

Nectar consisted of equal parts of fructose, glucose, and sucrose dissolved in water, with a hexose to sucrose ratio similar to that in natural nectars of glossophagine-pollinated plants (Baker et al. 1998). During a particular night one feeder in each cage received nectar from one pumping system, and the other feeder from the other system (Fig. 1). System 1 was always filled with 20% w/w concentration and the concentration in System 2 varied throughout the experiment. Thus during a single night the concentration offered at each feeder

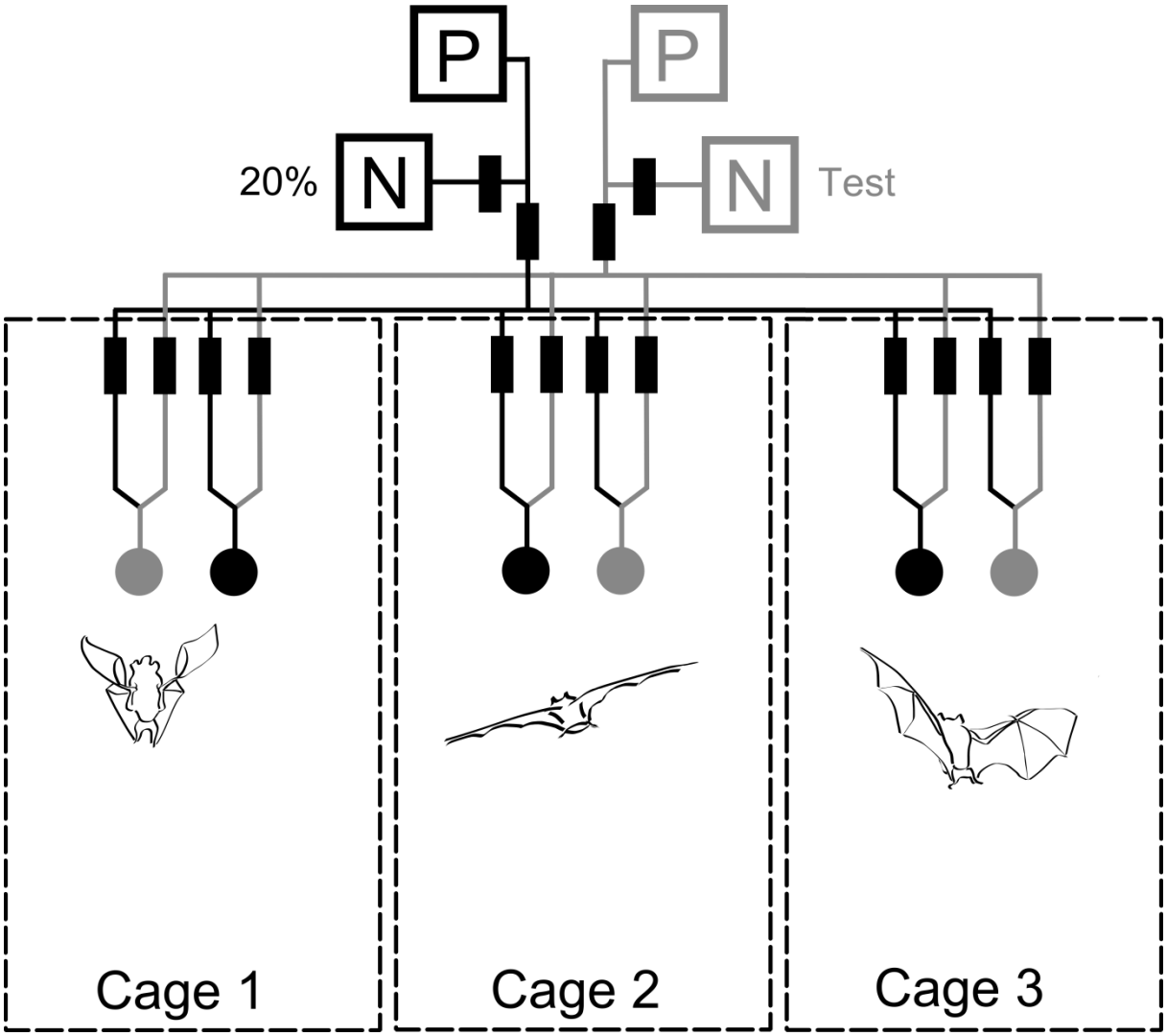

Figure 1. Schematic overview of the cage and feeder system. Two feeders (*circles*) were placed inside each cage (*boxes with dashed outlines*). Every feeder was connected via tubes (*continuous lines*) to two nectar pumping systems. One pump (*black P*) was connected to a 20% sugar solution reservoir (*black N*) and the other pump (*gray P*) was connected to the reservoir with the test concentration (*gray N*), which differed with test condition (Table 1). The flow of nectar was regulated with the pumps and pinch valves (*black rectangles*).

Experimental Schedule

Experiments were conducted consecutively with two groups of three animals each and each group was subjected to a series of 2AFC tests. The first group of three bats participated in calibration tests of the cage system for six nights before actual testing began (Table A1).

The three bats of the second group started with the experiment on the day of the transfer to the cages.

The two feeders in every cage gave different sugar concentration rewards during each experimental session. Every session lasted 12 hours and consisted of two phases: a forced alternation phase, and a choice phase. The alternation phase lasted for 100 visits (50 samplings per feeder) and ensured that the bats experienced both nectar concentrations equally. Strict alternation was achieved by blocking a feeder opening with the plastic guard after every visit. During the choice phase the plastic guards were automatically removed from both feeders until the end of the session so that bats could access both feeders freely. During the inter-session interval (ISI = 12 h) the lights were on and all feeders were inaccessible.

Of the two feeders in each cage one (standard feeder) always gave rewards with 20% w/w sugar concentration (632 mmol L^{-1} sucrose equivalents, Bolten et al. (1979)). The nectar concentration of the other feeder (test feeder) was systematically changed (Table 1) and ranged from 8 to 50% weight/weight (226 to 1796 mmol L^{-1} sucrose equivalents). The test concentrations were chosen to be symmetrical around the standard concentration of 20% with respect to their relative intensity value. I avoided concentrations higher than 50%, because for sugar concentrations above 52% the increase in viscosity is expected to cause a reduction in net energy gain (Kim et al. 2011). The sequence of test concentrations within both series was random. However, every condition was presented twice on consecutive nights on which the feeder positions for the test and standard concentration were exchanged (Fig. 1, black and gray feeders), as a control for positional biases. Since the cages were supplied with nectar from the same two pumping systems, the sequence of test conditions was equal for bats within the same group. In each cage, the choice of position for the test feeder on the first presentation of a particular condition was pseudorandom, with an equal number of left and right starting positions for the test concentration.

Data Analysis

Analysis was limited to the first one hundred visits of the free choice phase, in order to analyze choice after an equal number of samplings at both feeders. For each bat and each condition I calculated the *relative intensity* and *discrimination performance*. The relative intensity was calculated as the absolute difference between the two sugar concentrations, divided by the mean concentration (see Equation 2). As explained in the introduction, this measure of intensity is analogous to the Weber ratio of $\Delta(a)/a$ and captures the expectations

Table 1 Sequence of experimental conditions in the two subject groups

	Group 1		Group 2	
Sequence[a]	Test concentration[b]	Relative intensity[c]	Test concentration[b]	Relative intensity[c]
1	12.5	0.46	20	**0.00**[d]
2	18.6	0.07	30	**0.40**
3	25	**0.22**	8	0.86
4	29	**0.37**	18.6	0.07
5	8	0.86	13.3	0.40
6	21.5	**0.07**	50	**0.86**
7	32	**0.46**	16	0.22
8	30	**0.40**	13.8	0.37
9	13.3	0.40	32	**0.46**
10	20	**0.00**[d]	29	**0.37**
11	16	0.22	17	0.16
12	50	**0.86**	25	**0.22**
13	23.5	**0.16**	23.5	**0.16**
14	13.8	0.37	21.5	**0.07**
15	17	0.16	12.5	0.46

[a] Each condition in the sequence was tested twice on two consecutive nights, with the position of the test and standard feeder exchanged. In Group 1, the experiment was interrupted for 4 days between sequence 14 and 15 and in Group 2 for 8 days between sequence 7 and 8

[b] Sugar solution concentrations are given in % weight/weight. The concentration of the standard was always 20% w/w

[c] Relative intensity is calculated as the absolute difference between the test and standard concentrations divided by the average of the concentrations

[d] Numbers given in bold correspond to the HIGH data set (test concentrations equal to or larger than 20%). The rest of the numbers correspond to the LOW data set. The comparison with 20% was included in both data sets. As the R script for psychometric analysis did not accept the 0 intensity value, it was entered as 1.0×10^{-6} instead

that discrimination performance should increase with the difference (distance effect) and decrease with the mean magnitude of the two options (magnitude effect). Discrimination performance was calculated over the two presentations of the same condition as the number of visits to the higher sugar concentration feeder divided by the total number of visits ($N = 200$).

Psychometric Analysis

The data sets of each animal were separated into two subsets: the HIGH set contained the comparisons with concentrations larger than or equal to the 20% standard (Table 1) and the LOW set contained the comparisons with concentrations smaller than or equal to the 20% standard (Table 1). Psychometric analyses were performed on the two data sets from each individual and Weibull psychometric functions were fitted using the Bayesian algorithm proposed by Kuss et al. (2005) using R 2.10.1 (R Development Core Team 2009). As prior function for the lapse rate I chose a beta distribution (2;10). For the threshold I chose a normally distributed prior with a mean of 1 and a standard deviation of 0.5, and for the slope a log-normal prior with a mean of 2 and a standard deviation of 1. I performed 5000 Markov Chain Monte Carlo (MCMC) sampling runs with a leapfrog step size of 100. From the individual psychometric functions obtained using this method, I calculated the mean threshold, slope, and lapse rate. I then used paired t tests to compare the three parameters of the psychometric functions obtained for the HIGH and LOW data sets. The prediction based on Weber's law was that there would be no differences between the parameters from the two sets.

Weber's Law vs. Near-Miss to Weber's Law

In order to test whether the near-miss to Weber's law provides a better fit to observed data than Weber's law I individually fitted psychometric functions as Weibull sigmoid curves using the following equation (Kuss et al. 2005; Chapters 1–2):

$$P_a(x) = \frac{1}{2}\left[\pi_l + (1-\pi_l)\left[2 - \exp\left(-\exp\left(\frac{2sm}{\ln(2)}\left(\ln\left(\frac{x-a}{\left(\frac{x+a}{2}\right)^{\beta}}\right) - \ln(m)\right) + \ln(\ln(2))\right)\right)\right]\right] \quad (5)$$

where x is the larger and a is the smaller of the sugar concentrations of the test and standard feeders, m is the threshold, s is the slope at the threshold, π_l is the lapse rate, and β is the exponent from Equation 4. In all models x and a were the independent variables, discrimination performance was the dependent variable, and m, s and π_l were estimated parameters. Using Akaike Information Criterion (AIC) scores I compared non-linear models in which the parameter β was either fixed at one (reducing near-miss relative intensity to relative intensity) or estimated within the model. I used the non-linear least-squares function nls in R 2.15.0 (R Development Core Team 2012).

Reanalysis of previously published data sets

I reanalyzed data from sugar discrimination experiments with nectar-feeding bats, hummingbirds, bumblebees, and honeybees. When analyzing previously published work by other authors, I extracted numerical values from the published scatterplot figures using EasyNData (Uwer 2007) and converted the sugar concentration in percentage weight/weight units. In order to test whether the near-miss to Weber's law provides a better fit to observed data than Weber's law I analyzed the transformed data using the procedure described in the previous section. If the 95% confidence intervals for β in two different groups both spanned a convenient round number, this number was taken to be the β value of both groups. This was done because it is not otherwise possible to compare threshold and slope parameters for psychometric functions based on near-miss relative intensities with different β values. (Comparison of lapse rates can be done regardless of differences in β values.) Psychometric analyses on the different data sets were performed with the algorithm published by Kuss and coworkers (2005) using as independent variables the near-miss relative intensity values calculated with the rounded β values.

Nectar-feeding bats

The same data were used as presented in Chapter 1. Data from 23 different *G. commissarisi* individuals were pooled and analyzed together.

Hummingbird data

Reanalyzed data were from a study on the concentration preferences in different hummingbird species (Hainsworth and Wolf 1976). The food intake from two adjacent feeders was monitored at half-hour intervals in nine individuals from five different species. The positions of the low and high concentration feeders were exchanged every half hour, for a total of 6 to 12 half-hour intervals. The sucrose concentrations were in the range of 0.15 to 1.10 M (5 to 33% w/w), with fixed differences of either 0.05 M (1.7 % w/w), 0.10 M (3.4 % w/w), or 0.20 M (6.7 % w/w). Discrimination performance data were extracted from Figure 1 in Hainsworth and Wolf (1976).

Bumblebee data

The same data was used as presented in Chapter 2. Data were pooled over the three marked and all unmarked individuals and analyzed together.

Honeybee data

Reanalyzed data were from a study on the concentration preferences in the Italian honeybee (*Apis mellifera ligustica*) (Sanderson 2006). In these experiments 18 blue and 18 white feeders were used, randomly distributed within a 6 × 6 square array and the concentrations of the two feeder colors were systematically varied. There were 27 different concentration pairings (7 experiments × 4 treatments minus 1 treatment from the first experiment) for which relative visitation rates to the higher concentration feeders for different sets of 3-4 bees over 40 visits per bee per treatment were measured. The mean sucrose concentrations in the seven experiments were from 0.25 to 1.75 M (8.3 to 49% w/w), with differences between the two feeder colors of either 0 M (0 % w/w), 0.2 M (6.7 % w/w), 0.4 M (13.0 % w/w), or 0.6 M (19.1 % w/w). Discrimination performance data were extracted from Figures III though IX, Chapter 4 in Sanderson (2006).

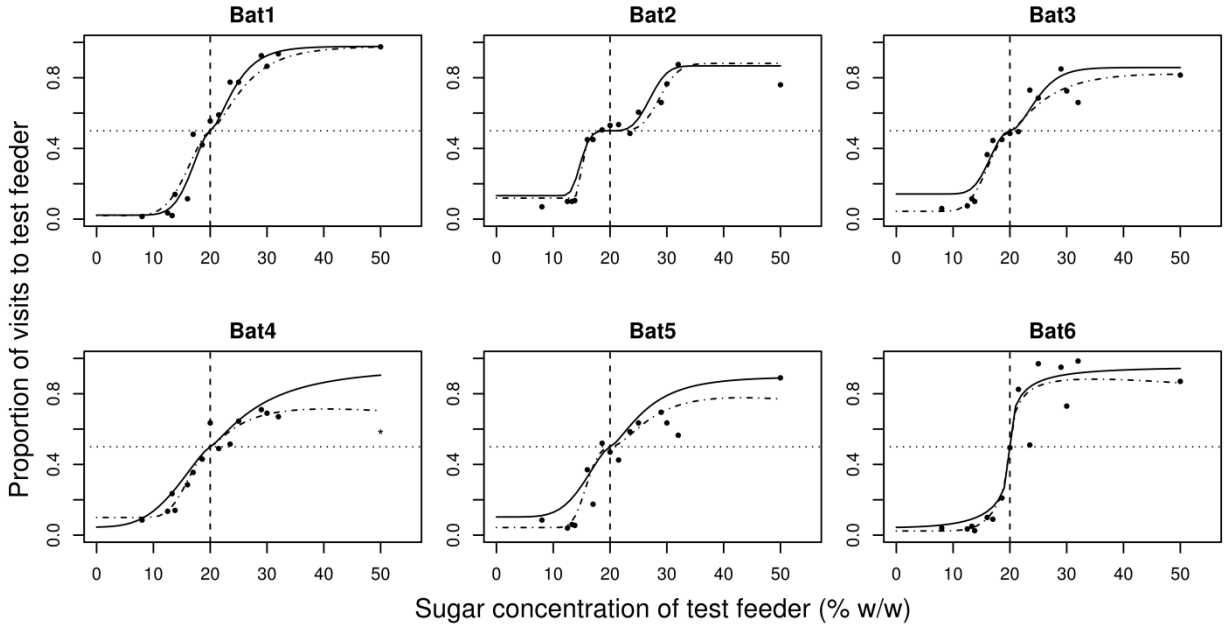

Figure 2. Discrimination performance versus concentration of the test feeder for the six experimental subjects. The sugar concentrations of the test options are given on the *abscissa*. *Black circles* represent the proportion of visits to the test feeder averaged over the two presentations of the same pairs of concentrations (Table 1). The *vertical dashed line* indicates the standard option (20% w/w). The *horizontal dotted line* indicates the chance level at 0.5. *Continuous lines* give the non-linear fit based on Weber's law model (Equation 5 with $\beta = 1$). *Dash-dotted lines* give the non-linear fit based on the near-miss to Weber's law model (Equation 5 with β as a free parameter). The datum for Bat 4 at 50% sugar concentration (*star*) was excluded as an outlier

Results

The discrimination performance of the bats varied with the magnitude of the test option. Bats either preferred the higher nectar concentration or showed no preference between the referent and standard feeders (Fig. 2). Contrary to the prediction based on Weber's law, expressing the differences between nectar concentrations in terms of relative intensity did not result in the same discrimination performances for the LOW and HIGH data sets in all animals (Figure 3). In the HIGH data set Bat 4 reached a maximum discrimination performance of 0.71 and at the highest intensity (i = 0.86) its discrimination performance actually dropped to 0.59. As these values resulted in high uncertainty of the psychometric function parameters, I excluded as an outlier the point at i = 0.86 from further analysis.

The average psychometric functions of the experimental subjects were significantly different for the LOW and HIGH data sets (Figure 4). The slope of the psychometric function from the HIGH data set (mean ± SE = 2.10 ± 0.39) was significantly shallower than the slope obtained from the LOW data set (3.65 ± 0.41, Paired $t(5) = 4.47$, $p = 0.007$). The lapse rate from the HIGH data set (0.18 ± 0.05) was higher than the lapse rate from the LOW data set (0.10 ± 0.02), but this difference did not reach significance (Paired $t(5) = -2.38$, $p = 0.06$). The

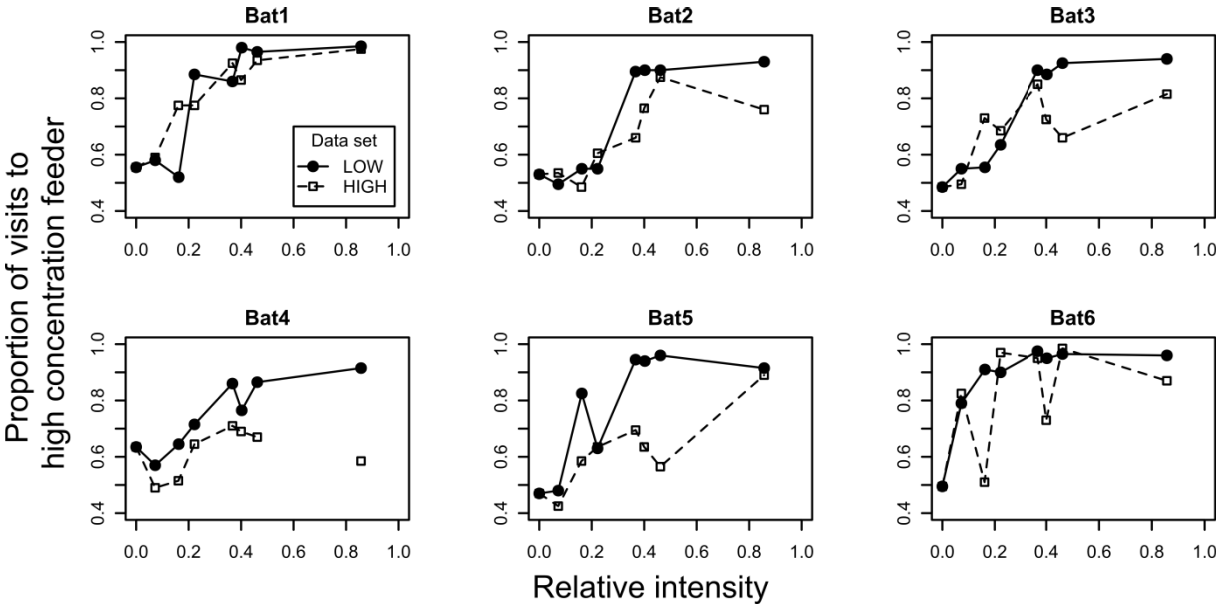

Figure 3. Discrimination performance as a function of relative intensity for the six experimental subjects. The same data as in Fig. 2 are plotted, but with relative intensity on the *abscissa* (see Methods and Table 1). *Black circles* represent the proportion of visits to the standard feeder (with concentration of 20% w/w) in choices when the test feeder had a lower concentration than the standard (LOW data set). *White squares* represent the proportion of visits to the test feeder in choices when its concentration was higher than that of the standard (HIGH data set)

thresholds of the two psychometric functions were 0.30 ± 0.07 and 0.21 ± 0.03 for the HIGH and LOW data sets, respectively, and did not differ significantly (Paired $t(5) = -1.42$, $p = 0.21$). (With the outlier from the data set of Bat 4 included, the mean ± SE of the slope, lapse rate, and threshold of the HIGH data set were 1.93 ± 0.49, 0.19 ± 0.05, and 0.40 ± 0.15, respectively. The resulting paired t test values for the comparison of the LOW and HIGH data sets were $t(5) = 5.71$, $p = 0.002$ for the slope, $t(5) = -2.67$, $p = 0.04$ for the lapse rate, and $t(5) = -1.38$, $p = 0.23$ for the threshold.)

For two of the six bats (Bats 1 and 6; Fig. 2; Table 2) Weber's law and the near-miss to Weber's law resulted in equally good fits. For the remaining four bats the near-miss to Weber's law was a significantly better model (Bats 2-5; Fig. 2; Table 2). The estimated average value (± SE) for the exponent in the near-miss to Weber's law was 2.44 ± 0.37 and was larger than one in all six bats.

Review of sugar discrimination in different nectar-feeding animals

In all of the analyzed data sets the estimates for the exponent β from Equation 5 statistically differed from one (Fig. 5; Table 3). In the vertebrate group of nectar-feeding animals β was estimated at 2.39 in *G. soricina*, 1.43 in *G. commissarisi*, and 2.09 in hummingbirds (Table 3). The 95% confidence intervals for these estimates spanned 2.0 in *G.*

Table 2 Model comparison between near-miss to Weber's law and Weber's law

	Near-miss to Weber's law		Weber's law		Model comparison		
	β[a]	AIC	β[a]	AIC	ΔAIC	F	*p*
Bat 1	-0.30 | 1.11 | 2.52	-32.59	1.00	-34.56	-1.97	0.022	0.885
Bat 2	0.54 | 1.81 | 3.57	-43.34	1.00	-35.71	7.63	9.901	0.008
Bat 3	1.53 | 2.37 | 3.22	-33.70	1.00	-28.86	4.83	6.393	0.026
Bat 4[b]	1.15 | 2.94 | 4.73	-31.47	1.00	-25.54	5.93	7.659	0.018
Bat 5	1.71| 2.72 | 3.72	-23.32	1.00	-14.30	9.02	11.893	0.005
Bat 6	0.14 | 3.70 | 7.25	-18.33	1.00	-18.16	0.17	1.747	0.211

In both models Equation 5 was fitted against observed individual discrimination performances. Lower Akaike Information Criterion (AIC) scores indicate a better fit of a model to the data, after penalizing for the number of estimated parameters. AIC scores can only be compared within rows but not between rows. ΔAIC gives the difference between the AIC scores for the model based on Weber's law and the model based on the near-miss to Weber's law. *F* and *p* values are based on one-way ANOVAs with 1 df

[a] The exponent β was fixed with value one in the Weber's law model and was estimated in the near-miss to Weber's law model. Values in the middle are average estimates and the values to the left and right are the 95% confidence interval limits

[b] One outlier was removed from the HIGH data set of Bat 4

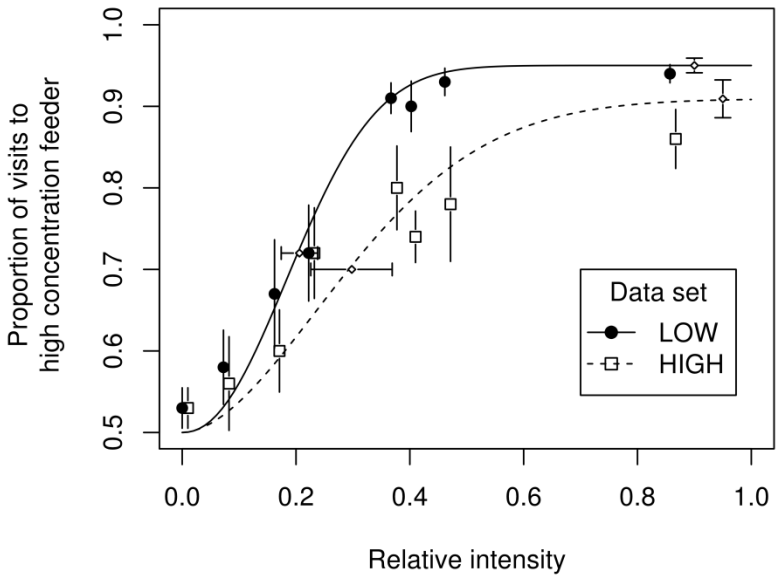

Figure 4. Psychometric curves for the LOW and HIGH data sets. The *abscissa* gives the relative intensity. *Black circles* represent the average proportion of visits to the standard feeder (with concentration of 20% w/w) over the six experimental animals in choices when the test feeder had a lower concentration than the standard (LOW data set). *White squares* represent the average proportion of visits to the test feeder over the six experimental animals in choices when the concentration of the test feeder was higher than that of the standard (HIGH data set). In order to prevent overlap in the graph, white squares are plotted with a horizontal jitter of 0.01 to the right. *Vertical bars* represent standard errors. The *continuous curve* represents the psychometric function with parameters (lapse rate, threshold and slope) averaged over the psychometric function parameters of the six experimental animals individually estimated from the LOW data set. The *dashed curve* represents the average psychometric curve obtained from the HIGH data set using the same procedure. The mean values of the threshold and upper asymptote for each curve are represented by *white diamonds*. *Whiskers* represent the standard errors

soricina and in hummingbirds, but neither of these intervals overlapped with the confidence interval estimated in *G. commissarisi* (Table 3). Thus, for psychometric analyses, β was set at 1.4 in *G. commissarisi* and at 2.0 in *G. soricina* and in hummingbirds. In the bees the estimate for β was 0.29 in *A. mellifera ligustica* and –0.04 in *B. impatiens* (Fig. 5; Table 3). The 95% confidence interval for β in *A. mellifera ligustica* did not span zero, but that of *B. impatiens* did. However, since both confidence intervals overlapped and spanned 0.3, in further psychometric analyses I set β at 0.3 in *A. mellifera ligustica* and in *B. impatiens*.

The estimates for the threshold, slope, and lapse rate for the psychometric functions of the different groups of animals were as follows: *G. soricina:* $m = 0.12$, $s = 4.4$, $\pi_l = 0.11$;

G.commissarisi: $m = 0.14$, $s = 9.7$, $\pi_l = 0.06$; Trochilidae: $m = 0.14$, $s = 4.3$, $\pi_l = 0.19$; *B. impatiens:* $m = 0.23$, $s = 4.1$, $\pi_l = 0.25$; *A. mellifera ligustica:* $m = 0.35$, $s = 1.9$, $\pi_l = 0.07$. The values for the lapse rates were in the range of 0.06–0.25 and were, as expected, somewhat higher than the typical lapse rates in human studies (0.0–0.10; Kuss et al. 2005). The psychometric functions are shown in Fig. 5.

Discussion

The results from this study (Figs. 2–4; Table 2) as well as the reanalysis of previously published data sets for different nectar-feeding animals (Fig. 5; Table 3) all support the near-miss to Weber's law as a better predictor of discrimination performance than Weber's law. This means that when the options within two sets of alternatives differ by the same Weber fraction the probabilities of choice for the each of the two options within one set of alternatives still changes as overall intensity increases. It is important to note exactly in which direction Weber's law fails to predict discrimination performance in the different animal groups. As explained in the introduction, the near-miss to Weber's law is a quantitative refinement of Weber's law introduced as an attempt to correct for the overestimation of the magnitude effect when applying Weber's law to data from acoustical discrimination tasks (Doble et al. 2003; Augustin and Roscher 2008; Augustin 2008, 2009). In my review of sugar concentration discrimination in bees the magnitude effect was also found to be weaker than predicted by Weber's law, since the exponent β was significantly lower than one (Table 3). However, the estimate for β was significantly higher than one in all vertebrates (Table 3). Thus, the magnitude effect in the vertebrates was actually stronger than predicted by Weber's law.

The estimate for the β exponent for bumblebees was zero (Table 3), suggesting the absence of the magnitude effect. However, the 95% confidence interval was quite broad and included the value estimated for honeybees (0.3, Table 3). Furthermore, the sugar concentrations tested with bumblebees were only in the range of 15-50% w/w and were probably too high to allow the detection of the magnitude effect. In the full honeybee data set the range of sugar concentrations was broader (1.7-55.5% w/w). When the sessions with concentrations below 10% w/w were removed from the honeybee data set, the estimate for β was also reduced to zero (not shown). I tentatively conclude from this analysis that the magnitude effect in bees is small and only detectable when a broader range of sugar concentrations is tested, including values lower than 10%.

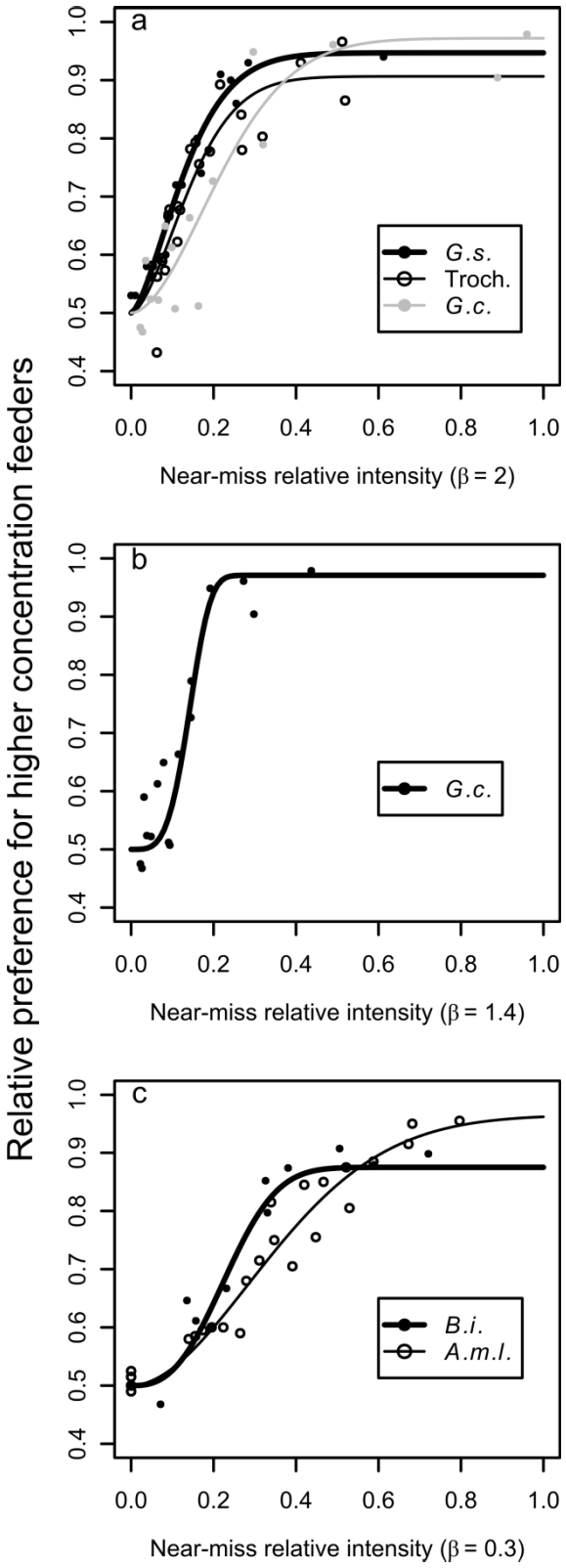

Figure 5. Psychometric curves based on near-miss relative intensities for different nectarivorous species. The *abscissa* gives the near-miss relative intensities as defined in equation (4) and scaled with different constant factors. *Symbols* give average discrimination performances measured in different experiments with different species. *Lines* give the fitted psychometric functions.

a Psychometric functions for hummingbirds (*thin black line, empty circles*) and *G. soricina* (*thick black line, black circles*). The exponent β in equation (4) was fixed at 2 and the scaling factor was 10. For comparison, the psychometric curve of *G. commissarisi* (*gray line, gray circles*) with assumed $\beta = 2$ is also shown. (Actual best-fitting β values are given in Table 3.) *G.s.* – *Glossophaga soricina*, this study; *G.c.* – *Glossophaga commissarisi*, see Chapter 1; Troch. – different Trochilidae species from Hainsworth and Wolf (1976).

b Psychometric function for *G. commissarisi* (*thick line, black circles*). The exponent β was fixed at 1.4 and the scaling factor was 1.

c Psychometric functions for bumblebees (*thick line, black circles*) and honeybees (*thin line, empty circles*). The exponent β was fixed at 0.3 and the scaling factor was 0.1. *B.i.* – *Bombus impatiens*, see Chapter 2; *A.m.l.* – *Apis mellifera ligustica*, Sanderson (2006)

As mentioned in the introduction, the psychometric function in animal studies estimates discrimination performance rather than the capacity for perceptual discrimination. Animals might perceive differences between the available options but distribute their visits

Table 3 Model comparison between near-miss to Weber's law and Weber's law in different nectar-feeding animals

Species	Near-miss to Weber's law	Model comparison with Weber's law			
	β[a]	AIC	ΔAIC	F	*p*
G. soricina	1.81 | 2.39 | 2.99	-153.3	-14.6	17.3	***
G. commissarisi	1.29 | 1.43 | 1.58	-385.2	-28.0	31.2	***
Trochilidae	1.59 | 2.09 | 2.59	-58.2	-23.2	38.5	***
B. impatiens	-0.51 | -0.04 | 0.55	-66.6	-8.0	10.3	0.004
A. mellifera ligustica	0.15 | 0.29 | 0.43	-96.2	-31.3	55.8	***

In both models Equation 5 was fitted against observed discrimination performances. Lower Akaike Information Criterion (AIC) scores indicate a better fit of a model to the data, after penalizing for the number of estimated parameters. AIC scores cannot be compared between rows. ΔAIC gives the difference between the AIC scores for the near-miss to Weber's law model and Weber's law model. *F* and *p* values are based on one-way ANOVAs with 1 df.

Sources: *Glossophaga soricina*, this study; *Glossophaga commissarisi*, see Chapter 1; different Trochilidae species from Hainswort and Wolf (1976); *Bombus impatiens*, see Chapter 2; *Apis mellifera ligustica*, Sanderson (2006).

*** $p < 0.001$.

[a] The exponent β was estimated in the near-miss to Weber's law model and fixed at one in the Weber's law model. Values in the middle are average estimates and the values to the left and right are the 95% confidence interval limits

more evenly between the alternatives, regardless of expected value. If we challenge the assumption that the probability to lapse (i.e. make a visit at random) is constant and independent from the presented stimuli, an alternative explanation for differences in performance and the observed magnitude effect could be the trade-off between exploitation and exploration. In other words, animals might achieve perfect perceptual discrimination (for options that are sufficiently different) but lapse more often when the costs of information-gathering are low, i.e., when food resources are rich or when animals are at high energetic states. However, if a richer environment promoted lapsing, then the *G. commissarisi* bats from the field study should have lapsed more often in the 5% vs. 20% and in the 15% vs. 30% conditions when average total sugar reward was higher than in the 5% vs. 10% condition (assuming equal perceptual discriminability under all conditions). The observed lapse rates showed the opposite pattern and were the highest in the poorest condition (0.04 in the 5% vs. 20% condition, 0.10 in the 15% vs. 30% condition, and 0.20 in the 5% vs. 10% condition (see Chapter 1, Fig. 2, black and gray points at relative intensities 1.20 and 0.67, respectively). The

variable lapse rate hypothesis cannot be discarded based on this counter-argument, but I consider it a less likely explanation of the observed patterns of discrimination performance.

In the remainder of this section I will discuss the differences in discrimination performance between the different groups of nectar-feeding animals and relate these differences to the nectar traits of plants pollinated primarily by vertebrate or by bee pollinators. Typical bat-pollinated and hummingbird-pollinated plants have dilute nectars with sugar concentrations of 13-18% w/w (Pyke and Waser 1981; von Helversen and Reyer 1984) and 23% w/w (Pyke and Waser 1981), respectively. Typical bee-pollinated plants on the other hand have nectars with higher sugar concentrations of 35% w/w (Pyke and Waser 1981). On the evolutionary timescale, bees and bee-pollinated plants predate vertebrate pollinators (Poinar and Danforth 2006) and transitions from insect pollination to vertebrate pollination are more common than vice versa (Thomson and Wilson 2008; Fleming et al. 2009). Based on these observations, it appears that transitions from bee to vertebrate pollination are associated with a decrease in nectar sugar concentration. A reasonable expectation is therefore that discrimination performance for sugar concentration may be different in bees and in vertebrates, with bees possibly better discriminating between higher concentrations. In general, better discrimination performance can be indicated by a lower lapse rate, lower threshold, and steeper slope. Next, I consider each of these three psychometric function parameters in turn.

The similar lapse rates in the different groups of animals suggest similar general motivational and explorative tendencies. As the lapse rates are fairly low, the psychometric functions in all animals are likely to give good approximations of the actual capacity for perceptual discrimination. The somewhat higher lapse rate in *B. impatiens* (π_l = 0.25) was probably overestimated because of the lack of sessions with very low concentrations. The threshold and slope can only be directly compared in groups with the same β estimate. As detailed in the introduction, β is the parameter that determines how strong the magnitude effect is with respect to the distance effect. Such comparison was possible between *G. soricina* and hummingbirds with β = 2 and between honeybees and bumblebees with β = 0.3 (assuming that the true value of β is similar in honeybees and bumblebees and that it was better estimated in *A. mellifera ligustica*). *G. soricina* had a psychometric function with a lower threshold and a steeper slope than the hummingbirds (Fig. 5a). This difference is consistent with bats often visiting flowers with even more dilute nectars than hummingbirds. However, the discrimination performance of hummingbirds might have been underestimated because performance was scored as food intake rather than as asymptotic visitation rates. It

has been demonstrated that the inclusion of the learning phase can shift the psychometric curve to the right and flatten it (Fründ et al. 2011). Furthermore, the two psychometric functions for bats and hummingbirds were more similar to each other than either of them was to the function fitted for *G. commissarisi* (Fig. 5a and 5b). The poorer discrimination performance of *G. commissarisi* could be due to the higher difficulty of the task, in which 24 feeders rather than two were available. In the bee group the psychometric function of the bumblebees had a lower threshold and a steeper slope than that of the honeybees (Fig. 5c). Again, the poorer performance of honeybees might be explained by the inclusion of the learning phase in the measure for discrimination performance. Nonetheless, the two functions were fairly similar (Fig. 5c).

Using Equation 5 with the appropriate fitted parameters for each group it is possible to extrapolate discrimination performances of different nectar-feeding animals in 2AFC tasks for a given standard option. This allows us to compare discrimination performances for groups with different β parameters (Figure 6). Bats and hummingbirds are predicted to outperform bees when the standard option is at 10% w/w and the referent option is either more dilute or more concentrated than the standard (Fig. 6a). In contrast, when the standard option is at 50% w/w and the referent option is lower than 50% w/w, bees are expected to outperform vertebrate nectar-feeding animals (Fig. 6c). The situation is intermediate with a standard at 25% w/w concentration; all animals are expected to be about equally good at discriminating referent options with concentrations lower than 25% w/w, but bees are expected to outperform bats and hummingbirds if the concentration of the referent option is higher than 25% w/w (Fig. 6b).

Thus, if Equation 5 accurately predicts discrimination performances, then bees do not simply outperform vertebrates. Instead the relative discrimination performance of different species is context-dependent: with high standards bees outperform vertebrates and with lower standards, vertebrates outperform bees. The mathematical explanation for this unexpected prediction lies in the strength of the magnitude effect (the value of β). Since the magnitude effect is much stronger in vertebrates, their initially better discrimination performance deteriorates faster with the increase in mean concentration, falling below the discrimination performance of bees. In summary, pollinators examined in this study are most sensitive to differences in sugar concentration in the typical ranges of the flowers they naturally pollinate.

The independently obtained estimates for the strength of the magnitude effect were similar within and different between groups of animal pollinators, suggesting that the small

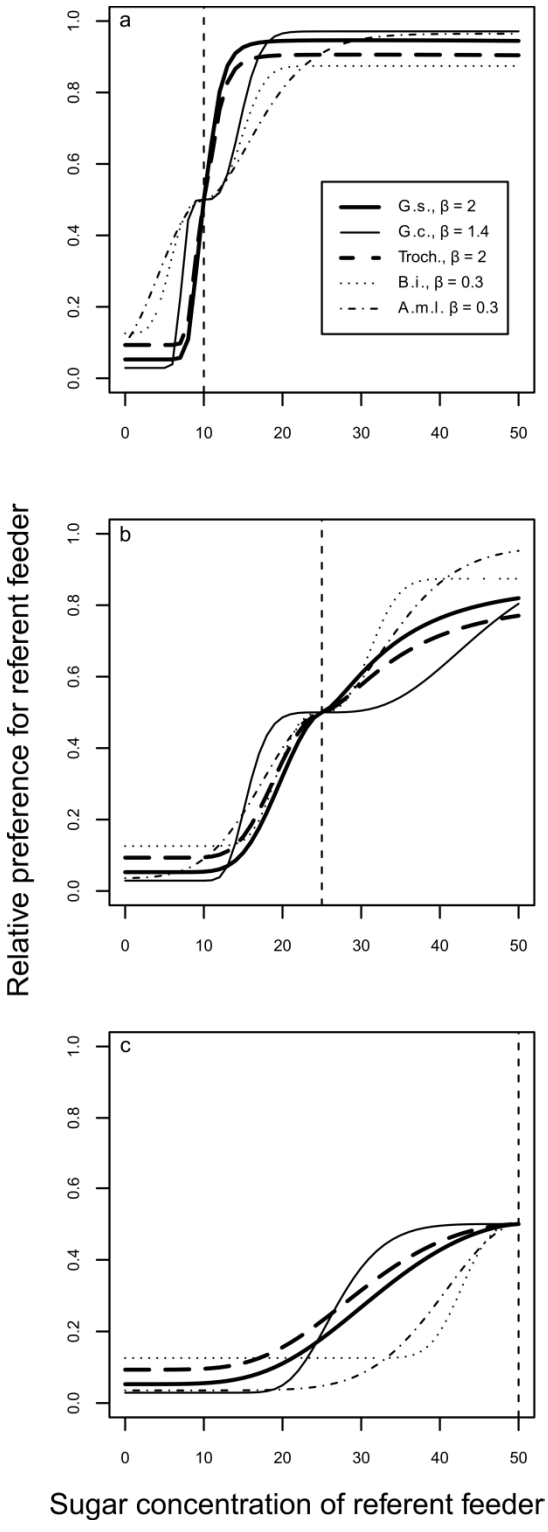

Figure 6. Predicted relative preference for nectar sources in a choice between a standard option and alternatives with different sugar solutions. The *vertical dashed line* passes through the sugar concentration of the standard option in *each panel*. Perfect discrimination performance would look like a step function at zero before and at one after the sugar concentration of the standard. The closer a curve lies to the *vertical dashed line*, the better the predicted discrimination performance.

a Standard option with 10% w/w concentration.

b Standard option with 25% w/w concentration.

c Standard option with 50% w/w concentration.

In *each panel* different *lines* give the predicted preferences for the alternative option of different nectar-feeding species: *G.s. – Glossophaga soricina* (*thick continuous line*), this study; *G.c. – Glossophaga commissarisi* (*thin continuous line*), see Chapter 1; Troch. – different Trochilidae species (*thick dashed line*) from Hainsworth and Wolf (1976); *B.i. – Bombus impatiens* (*dotted line*), see Chapter 2; *A.m.l. – Apis mellifera ligustica* (*dash-dotted line*), Sanderson (2006). The values of the exponent β are given in the legend

magnitude effect in bees and large magnitude effect in vertebrates may be the result from phylogenetic or morphological constraints. I expect the β estimates for yet untested insect and vertebrate pollinators to align to the pattern suggested by my analysis. On the other hand, I expect that as nectarivores become more specialized, there is directional selection pressure for the threshold of their psychometric function for sugar discrimination to become even smaller and for the slope to become steeper. These hypotheses

can be tested by subjecting more taxa of nectar-feeding animals to phylogenetic analyses of psychometric function parameters (Smith 2010).

As we have seen, the psychometric analysis of discrimination performance can be a useful tool for revealing sugar concentration values for which unexpected differences in discrimination performance are predicted between different nectar-feeding animals. Naturally, the predictive power of the near-miss to Weber's law model needs to be verified empirically.

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Chapter 3: Appendix

Table A1 Sequence of experimental conditions in the first six nights of the first subject group (*N* = 3 bats)

Sequence[a]	Test concentration[b]	Relative intensity[c]	Discrimination performance ± SE[d]
1	20	0.00	0.55 ± 0.07
2	16	0.22	0.61 ± 0.10
3	50	0.86	0.70 ± 0.11

These conditions immediately preceded the conditions given for Group 1 in Table 1. On nights 1–4 and on night 6, the same procedure was used as in the main experiment, except that after each visit during the free choice phase, the two feeders were closed and remained inaccessible for 30 seconds. This step in the procedure unnecessarily lengthened the duration of the free choice phase and was dropped in further experiments

[a] Each condition in the sequence was tested twice on two consecutive nights, with the position of the test and standard feeder exchanged

[b] Sugar solution concentrations are given in % weight/weight. The concentration of the standard was always 20% w/w.

[c] Relative intensity is calculated as the absolute difference between the test and standard concentrations divided by the average of the concentrations

[d] Discrimination performance was averaged over the two presentations of the same condition for each of the three bats, then the grand mean for the respective condition was calculated. The standard error was calculated over the mean values for each bat

CHAPTER 4: Behavioral syndromes and foraging performance in wild free-flying nectarivorous bats (*Glossophaga commissarisi*)

Abstract. Recent developments in animal personality research have revealed that individuals show specific suites of correlated behavioral responses (syndromes) that are consistent across different contexts and with time. The limitations of individual behavioral plasticity are potentially associated with differences in foraging efficiency and in fitness. Here, I present a field investigation on hypothesized behavioral syndrome structures of wild, free-flying nectarivorous bats (*G. commissarisi*) in the foraging context. Bats were automatically detected as they visited an array of 24 artificial flowers that delivered different nectar sugar concentrations. The individual behavioral measures derived from feeder visitation events were stable over the two-month observation period. I used structural equation modeling with model selection procedures to compare syndrome structures. The best supported model placed the behavioral measures in two independent groups, which I interpreted as the activity and shyness-boldness syndromes. Finally, I investigated the link between individual behavioral measures and measures of foraging performance. Under the experimental conditions there was no effect of activity on foraging performance. However, bats that visited fewer feeders were more likely to visit the higher sugar concentrations and performed better than bats that visited more feeders.

Introduction

Findings from the diverse fields of comparative behavioral biology, neurobiology, and psychology (Gosling 2001; Sih et al. 2004; Dingemanse and Réale 2005; Bell 2007; Réale et al. 2007) demonstrate that intra-specific behavioral variation is sometimes maintained in behavioral types: individual differences in behavioral responses remain stable with time and are consistent across various contexts. Such suites of correlated individual behaviors have been referred to as "animal personalities" (Gosling 2001), "behavioral syndromes" (Sih et al. 2004), "temperaments" (Réale et al. 2007), and "coping styles" (Koolhaas et al. 2007). It has been suggested that different behavioral types can be maintained in natural populations via frequency-dependent selection because different behavioral responses have similar fitness payoffs but are better adapted to slightly different environmental conditions (Benus et al. 1991; Sih et al. 2004; Dingemanse and Réale 2005; Penke et al. 2007; Wolf et al. 2007).

The idea that individuals tend to exhibit only a limited range of behavioral responses compared to the full behavioral repertoire of the species is also interesting from an evolutionary perspective. On the other hand, I believe that an investigation of the effects of individual behavioral differences to measures of resource use and decision making can also inform the research on foraging behavior and lead to more accurate models. My goals in this study goals in this study consist in: (i) identifying behavioral traits in the foraging context that exhibit variation among individuals and high consistency within individuals, (ii) testing whether the correlational patterns between these behavioral traits are consistent with previously hypothesized syndrome structures, and (iii) investigating the potential link between personality traits and individual measures of foraging performance.

I investigated these questions in a population of wild nectar-feeding bats (*Glossophaga commissarisi* Gardner). Bats from the genus *Glossophaga* exhibit considerable individual variation in foraging behavior (Winter and Stich 2005; Thiele 2006), which makes them suitable subjects for behavioral syndrome research. Compared to other members of the Glossophaginae subfamily (e.g. *Hylonycteris* or *Choeronycteris*) they are less specialized nectarivores with a broader diet (Tschapka 2004), which may result in a variety of foraging strategies adapted to different seasonal and local habitat conditions. Working with *Glossophaga soricina* in the laboratory I have performed exploratory analyses searching for personality traits that satisfy the conditions of being different among individuals but stable with time. I have noticed that individuals on average maintain the same level of activity (daily number of visits to feeders) and tend to exploit the same number of feeding locations (daily

number of different feeders visited) over observation periods lasting for several months (Chapter 4: Appendix). Visit duration (time spent in hovering flight in front of or clinging to a feeder) is another trait that showed consistency and it also tended to correlate negatively with activity, i.e. bats that made few visits made longer-lasting visits and vice versa (Chapter 4: Appendix). I hypothesized that the number of visits and visit duration might both be a part of the *activity* personality dimension (Réale et al. 2007). The measure of feeders visited I interpreted as an indicator of how much an animal invests in information-gathering while foraging or its tendency to form behavioral routines. This is the exploration-exploitation balance from reinforcement learning (Daw et al. 2006) and is a different concept than the exploration of novel environments or objects that is referred to as the *exploration-avoidance* continuum (Réale et al. 2007) in animal personality studies. In my experiments bats were tested on a daily basis in a familiar environment, which eliminates the element of novelty. Furthermore, studies in rodents indicate that routine formation and cue dependency are correlated with aggressive behavior (Benus et al. 1991; Koolhaas et al. 2007; Coppens et al. 2010). Consequently, we hypothesized that the measure of feeders visited is an indicator of an animal's *shyness-boldness* personality dimension (Réale et al. 2007) and as such would be independent from the other two measures. Consequently, I hypothesized that the measure of feeders visited is an indicator of an animal's *shyness-boldness* personality dimension (Réale et al. 2007) and as such would be independent from the other two measures.

I tested these hypotheses by analyzing a data set collected for the study described in Chapter 1, on the ability of *G. commissarisi* individuals to discriminate between different sugar nectar concentrations. In Chapter 1 foraging performance was assessed by fitting individual psychometric functions, relating the strength of preferring the higher concentration option to the relative intensity of the presented stimulus (i.e. perceived concentration difference between two sugar solutions). From the individual records of feeder visitation I determined the behavioral parameters number of visits per bout, visit duration, and feeders visited during nightly foraging. I then used these parameters to construct different hypothetical models of how these behavioral traits might contribute to a syndrome of individual personality. I compared hypothesized syndrome structures using structural equation modeling (SEM) and Akaike information criterion (AIC)-based model comparison (Dochtermann and Jenkins 2007; Dingemanse, Dochtermann, and Wright 2010; Dochtermann and Jenkins 2011). Finally, I used generalized linear models (GLMs) to investigate whether foraging performance measures could be predicted by individual personality traits.

Methods

I analyzed the behavior of 36 adult *G. commissarisi* bats (15 females and 21 males) at La Selva Biological Station, Province Heredia, Costa Rica. As described in Chapter 1 bats were caught by mist-netting, marked with RFID tags, measured, and released at the site of capture. As an indicator of a bat's size, I measured forearm length with calipers. Bats had free access to a patch of artificial flowers - a rectangular array of 24 computer-controlled nectar feeders suspended horizontally under a steel frame canopy. The distance between feeders in the same row was about 40 cm and the distance between rows, about 60 cm. The feeders delivered rewards of 55-60 µL on every visit. Half of them were supplied with nectar from one stock sugar solution, while the other half was supplied from a second stock solution with a different sugar concentration. During a single night an individual feeder's concentration was fixed, but the concentrations of the stock solutions were systematically varied throughout the experiment. The data set consisted of two separate experimental series of two-alternative free choice tests, with 12 feeders per option. During the first series, which lasted 18 days, the difference between concentrations was always 5 percentage points (from 5% vs. 10% to 45% vs. 50%). The difference between options during the second series was 15 percentage points (from 5% vs. 20% to 35% vs. 50%) and the series lasted for 14 days beginning one day after the end of the first series (for further details see Chapter 1).

Behavioral measures

The behavioral data collected during the experiments were the time stamped events of known duration of individually identified bats visiting specific feeders delivering rewards of known amount and sugar concentration. I present data from the 36 bats (out of a total number of 63 tagged bats) that made at least 24 feeder visits on at least 10 different days. The number of repeated measurements per individual was 23 ± 7.5 (mean ± SD). My sample of females consisted of 15 individuals, most of which were pregnant or lactating. As this number was too small for structural equation modeling analysis (see next section), I mainly focus on the behavior of the 21 male individuals. Three behavioral measures were calculated for each bat for every experimental day. Here I analyzed the complete record of events, in contrast to the analysis in Chapter 1, where only events between 20:00h and 3:00h were analyzed. The three behavioral measures were:

visits per bout – the total number of feeder visits divided by the number of bouts made by an individual bat during a single night. I split the foraging behavior of bats into bouts using 120 seconds as a bout break criterion (Fig. 1). If a bat made less visits than the number of feeders ($N = 24$), I excluded this day from the data set of this bat, in order to avoid a spurious positive correlation between number of visits per bout and number of *feeders visited* (see below).

visit duration – the mean of all visit durations (in milliseconds) made by a bat. Longer durations of several seconds indicate a tendency to make clinging visits instead of the brief hovering visits, which normally lasted less than a second. However, there is no clear threshold duration value that separates the two behaviors.

feeders visited – the total number of different feeders visited by an individual bat (ranging from 1 to 24).

Behavioral consistency

I expected the differences among bats for behavioral measures related to personality traits to remain stable over time, from the first to the second series. Two of the 36 bats (one male and one female) were not detected during the first series. I therefore excluded these animals and calculated the mean behavioral measures for the remaining 14 females and 20 males separately over the first and second series. I predicted a positive correlation between the data from the first and the second series and therefore used 1-tailed significance tests. For normally distributed measures I used Pearson's correlations and for non-normally distributed measures I used Spearman's rank correlations. I also performed repeatability analyses (Bell et al. 2009) separately for males and females on the data collected from all 36 bats. I used univariate random intercept models (Hadfield 2009; Dingemanse and Dochtermann 2013; *MCMCglmm* package in R 3.0.0, R Development Core Team 2013).

SEM analysis

I compared *a priori* hypotheses about syndrome structures using SEM and AIC-based model comparison with AMOS 5.0 (SPSS, Chicago; Dochtermann and Jenkins 2007, 2011;

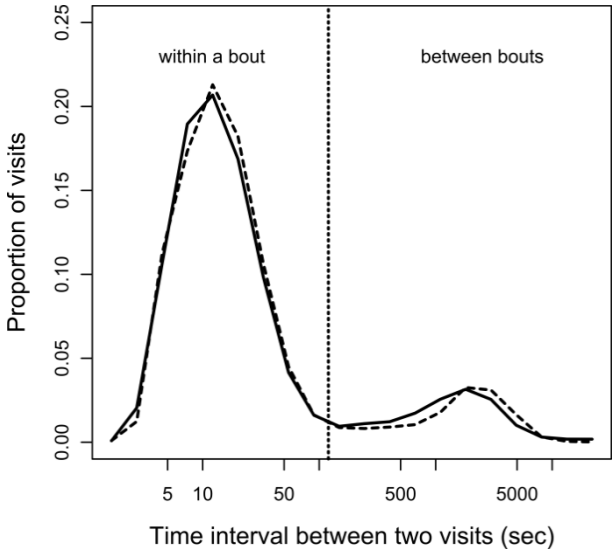

Figure 1. Bats' foraging behavior was organized in clear bouts. In both the first (*continuous line*, *N* = 34 bats) and the second (*dashed line*, *N* = 36 bats) experimental series the intervals between two visits had bimodal distributions when logarithmically transformed. Both curves are based on the cumulative visits of all bats present. I selected the time interval of 120 seconds (*vertical dotted line*) as a criterion to distinguish visits within a bout from visits between bouts

Dingemanse et al. 2010). This SEM approach has been shown to be applicable for data sets with small sample sizes that are often found in field behavioral studies on vertebrates (N ~ 20, Dochtermann and Jenkins 2011). I used the mean behavioral measures of all 21 male bats over the complete data set (including the first and the second series) to construct models of hypothesized syndrome structure (Fig. 2). The goodness of fit of the models was compared using AIC scores. For the AIC calculations I used likelihood discrepancies based on 1000 parametric bootstraps. A lower AIC score indicates higher explanatory power and the model with the lowest score was considered to represent the behavioral syndrome structure of *G. commissarisi*. Following Dochtermann and Jenkins (2007), I considered models with ΔAIC>2 (i.e. with AIC scores differing by more than 2 from the score of the model with the lowest AIC score) to be statistically unsupported.

Behavioral syndrome structure

Model 1. Behavioral independence. This was the null model with assumed lack of relationship between the behavioral measures.

Model 2. A link between visits per bout and visit duration (figure 2, only path "a" activated), both interpreted as indicators of the *activity* dimension (Réale et al. 2007). Feeders visited is independent from the other two measures and may be an indicator of the animals' *shyness-boldness* dimension (Koolhaas et al. 2007).

Model 3. A link between visits per bout and feeders visited (figure 2, only path "b" activated). In this model visit duration is independent from the other variables and not an indicator of any particular personality dimension, whereas visits per bout and feeders visited both reflect the *activity* dimension.

Model 4. A link between visit duration and feeders visited (figure 2, only path "c" activated), both interpreted as indicators of the *shyness-boldness* dimension. The rationale behind this hypothesis is that an animal might make visits to a higher number of different feeders to sample their nectar concentration and abort the visits prematurely when the concentration is of the lower type. In this model visits per bout is the sole indicator of the *activity* dimension.

Model 5. Full domain-general syndrome with all measures indicators of the same dimension, (e.g. *activity*).

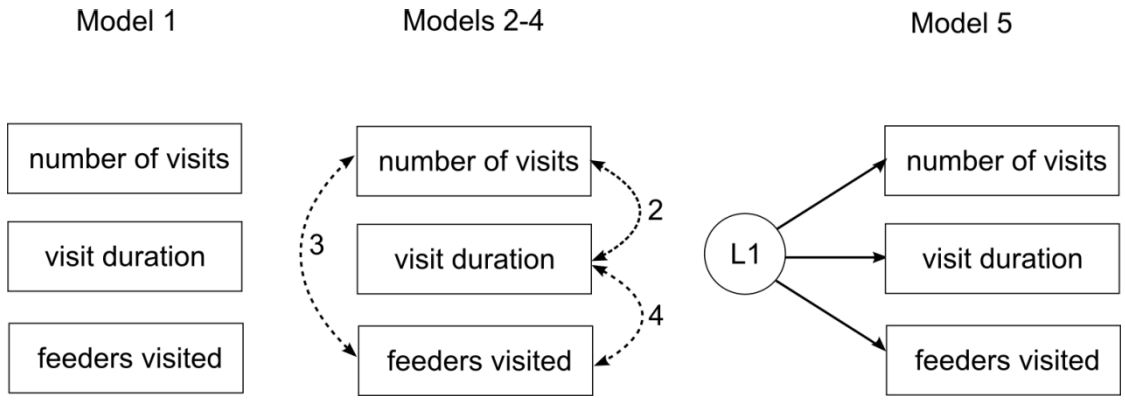

Figure 2. Models (1-5) of different syndrome structures. See "Behavioral syndrome structure" for model descriptions and Table 2 for results from model comparison. *Continuous unidirectional lines* represent a causal relationship from a latent variable (*L1*) to behavioral measures. *Dashed bidirectional arrows* represent correlations between behavioral measures expressed in particular syndrome structures. In model 2 only path '2' is active, in model 3 only path '3' is active and in model 4 only path '4' is active.

Foraging performance

I used two different measures of foraging performance, bouts to criterion and lapse rate, both calculated from the four nights on which the two most extreme differences in sugar concentrations were presented to the bats (5% vs. 20% and 10% vs. 25%). These pairs of sugar concentrations were chosen because they were associated with the highest, nearly perfect discrimination performance by bats (Chapter 1). Under these conditions the effect of individual differences in perception of sugar concentrations is minimized, so that differences in learning rates and non-perceptual error rates can be better estimated.

bouts to criterion – the number of bouts a bat made until the average proportion of visits to the higher sugar concentrations over the last two bouts was 0.9 or higher. All 21 males reached this criterion on every night except for one bat on one night and the final score was averaged over the four nights. (Two bats were only present on two nights and two other bats were present on three of the four nights.) All else being equal, this measure indicates that a bat is faster in avoiding options with lower sugar concentrations and therefore has a higher energy intake per visit. I expected bats with a higher number of visits per bout to reach the criterion in a smaller number of bouts. I also expected the stable experimental conditions (fixed sugar concentrations and volumes during a night) to favor bats with more routinized behavior that visit a small number of feeders, i.e. I expected a lower number of feeders visited to lead to a lower number of bouts to criterion. I had no prior expectation for the relationship with visit duration.

lapse rate – a measure of the relative frequency of errors (in this case, visits to the low concentration feeders) due to factors of a non-perceptual nature, e.g. information-gathering or exploratory behavior. It is calculated from asymptotic choice behavior, as one minus the proportion of visits to the higher sugar concentrations (discrimination performance) multiplied by two. As bats needed on average three bouts to reach the criterion of 0.9 (see previous paragraph), I calculated discrimination performances by pooling for each bat all visits after the fifth bout from all four nights. The rationale behind this measure rests on the assumption that a bat has a constant probability to lapse (select a feeder at random with respect to its concentration). Since the frequencies of the two feeder types were equal, the deviation of the discrimination performance from one represents only the half of the lapses that were visits to low concentration feeders. Bats visiting a few feeders were expected to follow a routine that they seldom break, thus having lower lapse rates. As lapse rate is a

measure calculated from asymptotic behavior, I expected it to be unaffected by visits per bout. I had no prior expectation for the relationship with visit duration.

I fitted two GLMs with bouts to criterion and lapse rate as dependent variables and visits per bout, visit duration, and feeders visited as independent variables. In the model for bouts to criterion I used the Gaussian family with the identity link function and in the model for lapse rate I used the quasibinomial family with the logit link function. Statistical analysis was carried out using R 3.0.0 (R Development Core Team 2013).

Results

According to my estimates, some 50–80 *G. commissarisi* bats foraged simultaneously during the two experimental series, making on average one visit per minute per individual. The behavioral consistency of all three measures in the 36 marked individuals was high, as indicated by the significant positive correlations between data from the first and from the second data set for visits per bout (males: Spearman's $\rho = 0.69$, 1-tailed $p < 0.001$, $N = 20$; females: $\rho = 0.82$, 1-tailed $p < 0.001$, $N = 14$), visit duration (males: Pearson's $r = 0.83$, 1-tailed $p < 0.001$, $N = 20$; females: $r = 0.84$, 1-tailed $p < 0.001$, $N = 14$), and feeders visited (males: $r = 0.48$, 1-tailed $p = 0.014$, $N = 20$; females: $r = 0.74$, 1-tailed $p = 0.001$, $N = 14$). Similarly, the repeatability estimates were high for visits per bout (males: $R = 0.49$, 95% credibility interval = 0.35, 0.69, $N = 21$; hereafter reported as $0.35 \leq 0.49 \leq 0.69$; females: $0.35 \leq 0.53 \leq 0.74$, $N = 15$), visit duration (males: $0.54 \leq 0.69 \leq 0.82$, $N = 21$; females: $0.59 \leq 0.70 \leq 0.86$, $N = 15$), and feeders visited (males: $0.19 \leq 0.39 \leq 0.55$, $N = 21$; females: $0.48 \leq 0.73 \leq 0.87$, $N = 15$).

Males did not differ from females in the number of visits per bout (Welch's *t*-test: $t = -0.71$, df = 27.6, $p = 0.48$) or in visit duration (Welch's *t*-test: $t = 0.19$, df = 32.7, $p = 0.85$). However, females visited on average more different feeders (mean ± SD = 11.55 ± 3.13, $N = 15$) than males did (mean ± SD = 7.81 ± 2.12, $N = 21$; Welch's *t*-test: $t = -3.89$, df = 22.8, $p < 0.001$; Table 1). Forearm length did not correlate significantly with visit per bout (males: $\rho = -0.10$, $p = 0.67$, $N = 21$; females: $\rho = 0.13$, $p = 0.64$, $N = 15$), visit duration (males: $\rho = -0.13$, $p = 0.56$, $N = 21$; females: $\rho = 0.13$, $p = 0.63$, $N = 15$), and feeders visited (males: $\rho = 0.05$, $p = 0.84$, $N = 21$; females: $\rho = 0.18$, $p = 0.52$, $N = 15$).

Table 1. Pearson correlation coefficients for behavioral parameters of the 36 bats with uncorrected *p*-values in parentheses. Values in bold on main diagonals give mean measures with standard deviations in parentheses. The value for visits per bout in males is reverse transformed

	visits per bout	feeders visited	visit duration (ms)
Males (N = 21)			
visits per bout	**6.76 (1.47)**		
feeders visited	0.09 (0.71)	**7.81 (2.12)**	
visit duration	–0.28 (0.22)	0.01 (0.96)	**664 (183)**
Females (N = 15)			
visits per bout	**8.23 (3.55)**		
feeders visited	0.18 (0.52)	**11.55 (3.13)**	
visit duration	–0.66 (0.007)	0.24 (0.39)	**653 (156)**

Table 2. Comparison of the six candidate models using Akaike Information Criterion (AIC). *K* is the number of estimated parameters in a model. Model discrepancy is based on bootstrap (*N* = 1000) maximum-likelihood analyses. Smaller AIC values indicate a better fit of a model to the data, after penalizing for the number of estimated parameters (*k*). ΔAIC gives the difference between the current and top model AIC values. A difference larger than two corresponds to models lacking in explanatory power relative to the top model

Model	*k*	Model discrepancy	AIC	ΔAIC
Visits per bout linked to visit duration (model 2)	4	2.42	11.16	0.00
Behavioral independence (model 1)	3	5.21	11.21	0.80
Visits per bout linked to feeders visited (model 3)	4	4.03	12.03	1.61
Feeders visited linked to visit duration (model 4)	4	4.17	12.17	1.75
Full-domain general syndrome (model 5)	6	1.00	13.00	2.58

The mean behavioral measures over the complete data set differed between the 21 male individuals (Table 1, main diagonal). According to Shapiro-Wilk tests the distributions of the behavioral measures visit duration (W = 0.956, p = 0.44) and feeders visited (W = 0.916, p = 0.07) did not differ from normality. The distribution of visits per bout deviated significantly from normality (W = 0.894, p = 0.03). Normality was achieved through a natural logarithm transformation (W = 0.965, p = 0.61).

Table 3. Summary of generalized linear model (GLM) statistics with number of bouts to criterion (Gaussian family with identity link function) and lapse rate (quasibinomial family with logit link function) as dependent variables

Behavioral measure	Bouts to criterion				Lapse rate			
	t value	*p*	Estimate	SE	*t* value	*p*	Estimate	SE
visits per bout	–0.86	0.40	–0.33	0.38	–0.54	0.96	–0.004	0.066
visit duration	–0.05	0.96	-4×10^{-5}	8×10^{-4}	0.40	0.70	-7×10^{-5}	6×10^{-5}
feeders visited	2.57	0.02	0.17	0.07	4.19	0.0006	0.35	0.08

In males the correlation coefficients between the behavioral measures were weak (Table 1), with the strongest correlation between visits per bout and visit duration ($r = –0.28$). (Correlation probability values were not explicitly considered; instead I used AIC values for model comparison). The direction of the correlation is consistent with the prediction that bats making many visits also make visits with shorter durations. There were no correlations between visits per bout and feeders visited ($r = 0.09$, Table 1), and between visit duration and feeders visited ($r = 0.01$, Table 1).

SEM analysis of behavioral syndrome structure

From the five different models tested (Fig. 2), the model in which number of visits was linked to visit duration had the lowest AIC score and best explained the observed data (model 2, Table 2). However, due to ΔAIC scores smaller than 2, four of the five tested models, including the model of behavioral independence, could not be statistically distinguished from each other (models 1–4, Table 2). Since the remaining model had a ΔAIC score larger than two (model 5, Table 2), I could reject it as statistically unsupported.

Relationship between behavioral measures and measures of foraging performance

The GLMs revealed that bats that visited more feeders on average needed a higher number of bouts to reach the criterion of 0.9 discrimination performance and had higher lapse rates (Fig. 3; Table 3). Visits per bout and visit duration did not affect either measure of foraging performance (Table 3).

Discussion

Consistent with my observations in the laboratory, the wild *G. commissarisi* from this study exhibited individual behavioral consistency in the number of visits per bout they made to a patch of artificial flowers, their mean visit duration, and the number of different feeders they visited. The individual behavioral differences were not related to differences in body size (from forearm length). In males the behavioral consistency could not be explained with a full domain-general syndrome, but more likely results from two or three distinct personality dimensions (Table 2). The model that best explained the results had visits per bout and visit duration linked together as indicators of the *activity* syndrome (Réale et al. 2007), but it could not be statistically distinguished from the model of full behavioral independence (Table 2). However, the same correlational pattern was also present and even stronger in female individuals (Table 1), which makes behavioral independence a less likely explanation.

Concerning the pattern of resource exploitation, some bats consistently visited only a few of the available feeders, whereas others spread their activity over more than half of the array (range of mean number of feeders visited: 3.5-17.8; Table 1). I interpret this difference as a difference in the degree of behavior routinization and the measure of number of feeders visited as an indicator of an animal's *shyness-boldness* dimension (Réale et al. 2007). Consistent with this interpretation, bats visiting more feeders were also the bats that showed the lower foraging performance scores (Table 3), presumably by making more visits at random and therefore more often visiting low sugar concentration feeders. The feeders always delivered rewards and the sugar concentrations of their nectars were stable within nights and only varied from night to night, thus favoring the development of routines and penalizing unnecessary information-gathering. In remains to be shown in future studies that number of feeders visited is linked to other behaviors on the *shyness-boldness* continuum (responses to non-novel risk situations; Réale et al. 2007), e.g. delay to resume foraging after a perceived predator attack.

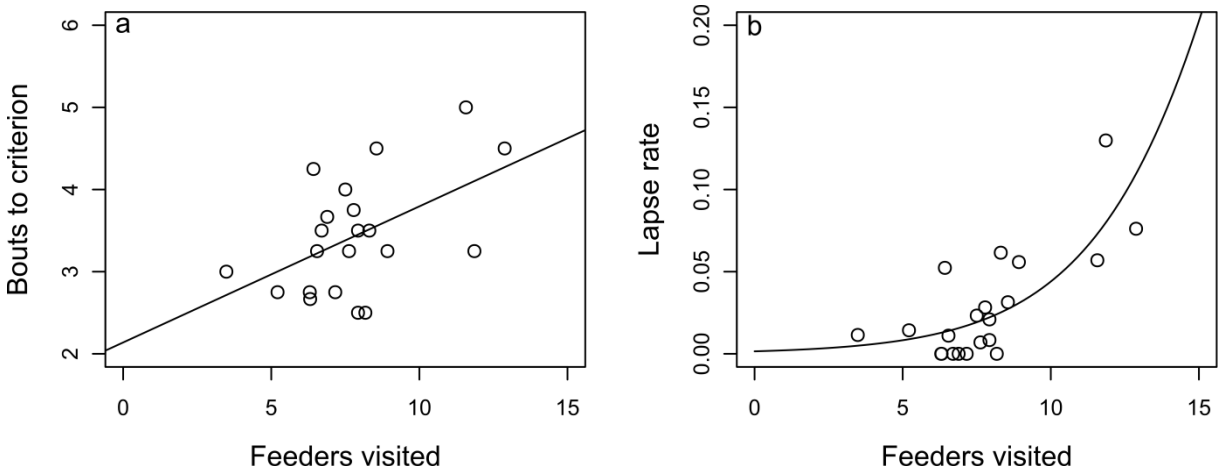

Figure 3. Relationship between foraging performance parameters and the number of feeders a bat visited. **a** Generalized linear model (GLM) fit of the model with feeders visited as independent and bouts to criterion as dependent variable. **b** GLM fit of the model with feeders visited as independent and lapse rate as dependent variable

These results provide some support to the hypothesis that different behavioral types may be better adapted to different environmental conditions, more specifically, to different resource qualities and distributions. Though I did not assess fitness directly, foraging efficiency has been demonstrated to be positively correlated with fitness (Ritchie 1990; Lemon 1991). Under conditions of higher reward uncertainty I would expect bats that invest more in information-gathering to have better chances of detecting the locations of more profitable sources of nectar. On the other hand, especially at flowers with high nectar secretion rates, the behavior of foragers with more routinized behavior may confer fitness benefits through "monopolization by exploitation". (I suggest this expression as a more general term for the "defense by exploitation" behavior described in Paton and Carpenter 1984 and Ohashi and Thomson 2005.) Foragers employing this strategy maintain high activity rates and exploit a limited number of replenishable food sources therefore keeping the mean resource standing crops low. This can reduce resource competition, because it can cause competitors using different strategies to perceive the same food sources as unprofitable and leave to forage elsewhere.

The different foraging strategies of the bats also have important implications for the fitness of the plants they pollinate. For example, bats with different propensities to make revisits to the same plant or flower can exert very different selection pressures on plants, especially if these are self-incompatible. Thus, both the quality and quantity of pollination service provided by a pollinator may depend on its behavioral type.

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Chapter 4: Appendix

The repeatability of foraging behavior in *Glossophaga soricina*

Abstract. Animal personality research has revealed that individuals show specific suites of correlated behavioral responses that are consistent across different contexts and with time. Searching for personality traits that would satisfy these conditions, I investigated the repeatability of foraging behavior in nectar-feeding bats. Over a three-month period and under two experimental conditions I presented six *Glossophaga soricina* individuals foraging in a group with an array of artificial flowers differing in nectar productivity. Behavioral parameters were recorded on a daily basis and exploratory analyses showed distinct differences among individuals. Over the whole duration of the two experiments most behavioral measures had repeatabilities higher than 0.5. Discrimination functions trained on data from the first experiment and used to classify by individual the cases from the second experiment achieved a hit ratio of 69% ($p < 0.001$). The measures that were most useful in distinguishing among individual bats and had the highest repeatabilities (>0.7) could be tentatively interpreted as pertaining to the *activity* and *shyness-boldness* behavioral syndromes.

Introduction

Suits of correlated behaviors that remain constant with time and across different situations are referred to as “animal personalities” (Gosling 2001), “behavioral syndromes” (Sih et al. 2004), and “temperaments” (Réale et al. 2007). A standard methodology for identifying behavioral syndromes has been to test a number of individuals on a multitude of different tasks and observe the general patterns of correlation between the variables using principal component or factor analysis, among other methods. However, variability in behavior may reflect current state differences that are not necessarily fixed for life and can therefore be explained without assuming that individuals belong to different behavioral types (Bell et al. 2009; Chervet et al. 2011). The case for behavioral syndromes can only be made for behaviors with a high repeatability, that is, behaviors for which variation is higher between individuals than within individuals (Lessels and Boag 1987; Bell et al. 2009).The repeatability of individual behavioral scores can be investigated with the so called test-retest procedure, in which individuals are scored on the same tasks at some later point(s) in time.

The limited behavioral plasticity of behavioral types implies that when comparisons are made across different contexts the relative levels rather than the absolute scores of the behavioral responses should remain consistent (Sih et al. 2004). This approach is in contrast with the standard experimental protocols, according to which different individuals should be used in different experimental treatments. However the sacrifice of statistical independence resulting from reusing the same individuals across contexts allows precisely for the quantification of the carryover effects that are usually avoided (Sih et al. 2004).

In this study I monitored the foraging behavior of six individuals of nectarivorous bats (*Glossophaga soricina*) foraging as a group over several months and performed an exploratory search for behavioral measures that would satisfy the aforementioned conditions of being different among individuals but stable with time and across test conditions in a foraging context. I chose previously gathered data sets from experiments on foraging choice. The conditions were designed to mimic tasks bats encounter in their natural environment, where they forage in mostly familiar surroundings and compete with each other for flower nectar, which is a limited but renewable resource. In Experiment 1 the experimental environment was modeled as a patch of flowers that differed in their nectar productivities. In Experiment 2, the complexity of the environment was increased by allowing the nectar concentrations of the artificial flowers to differ as well.

Materials and Methods

Bats

I monitored the foraging behavior of six Pallas's long-tongued bat (*Glossophaga soricina*) individuals, three males and three females, bred in captivity at Bielefeld University. All bats were adult, older than one year of age. Individuals were marked with unique Radio Frequency Identification tags (RFID) using silicon necklaces. Treatment of the experimental animals complied with the national laws on animal care and experimentation, under license of Veterinäramt Bielefeld.

Artificial flowers

The same set-up was used as described in Chapter 1, with few modifications. Twenty-four flowers were mounted on a vertical rectangular frame, forming a 4 × 6 array with about 40-cm horizontal and vertical distances between feeders. The distance between the lowest row and the ground was about 60 cm. The whole frame was positioned along one of the walls of the experimental room (5.4 × 4.7 m).

Reward schedule

In order to mimic the variation in nectar volume bats experience in nature, we assigned virtual "nectar accounts" to every feeder. The current state of the account determined the amount of nectar a bat could maximally receive as a reward upon making a visit. During the nightly experiments the amount in this account increased continuously at a constant rate, the nectar production rate being the same as the case in a natural flower. The maximum reward a feeder actually delivered was 54 μL of nectar during each single visit by a bat. If the account balance was less than 54 μL, the bat received the full amount remaining in the account. Feeders delivered no rewards until at least 4.5 μL of nectar had accumulated. Thus feeders could deliver 13 possible reward volumes ranging from 0 to 54 μl, with a common difference of 4.5 μL.

Nectar Concentrations

Nectar consisted of equal parts of sucrose, glucose, and fructose dissolved in water. The two pumping systems contained nectars with two different sugar concentrations, 10% and 50% (weight/weight). The sugar concentrations delivered to each feeder could be regulated through adjusting the ratio of the volumes of the two reward amounts delivered by each syringe pump. The mixing algorithm allowed for a spectrum of 21 different concentrations from 10 up to 50%, with a common difference of 2%. The accuracy of the mixing process had a SD of ± 3.5% units, estimated from experimentally delivered volumes using a hand-refractometer for measuring sugar concentrations. During a single night, the concentration offered from each single feeder was fixed and did not change. In order to prevent bacterial and fungal growth inside the tubing systems these were rinsed with warm water and a 70%-ethanol solution every 3-4 days. The more infection-prone 10% stock solution was always kept in a refrigerator at about 4 ºC.

Pre-training and general experimental conditions

Bats were transported to the experimental room about a month before the beginning of the experiments, so that the animals had ample time to become familiarized with the feeder array. The climatic conditions in the experimental room were 22 °C and approximately 60% humidity. Light conditions were LD 12:12 and the foraging sessions coincided with the scotophase. The feeder array was always present in the experimental room, but it was active only during the foraging sessions. Virtual nectar secretion was initiated an hour before the onset of the scotophase and continued for 13 hours, so that at the beginning of the foraging session each feeder had already accumulated some nectar in its virtual account. Nectar production rates were pseudo-randomly assigned to each feeder before each foraging session. I fixed nectar concentration at 18% (weight/weight) by using only one of the pumping systems and filling its reservoir with premixed stock nectar. Food provisioning occurred in a closed system where animals covered their daily energy expenditures almost exclusively by foraging on the feeders. In addition, bats received about 9 g of bee-collected commercial flower pollen (Type 9015, Friedrich Wienold), and a mixture of 1.2 g nectar concentrate for hummingbirds (Nektar Plus, Nekton) and 1.8 g human infant formula (Alete Folgemilch 2, Nestle) diluted in 6 ml of water. These supplementary nutrients were placed in open dishes on a raised platform in the experimental room 30 min before the onset of the scotophase and remained there until the following day.

Experiment 1

The experiment lasted for 15 days. The range of possible nectar production rates for each flower was from 90 to 450 µl h^{-1}, with a common difference of 18 µL h^{-1}. The nectar production rates were pseudo-randomly assigned to the different feeders and the mean nectar production rate of the whole 24-feeder array differed with daily foraging session (mean ± SD: 328.5 ± 29.2 µL h^{-1} SD, N = 15 days).

Experiment 2

This experiment took place 98 days after Experiment 1 and lasted for 16 days. Between Experiment 1 and Experiment 2 the whole group of six bats remained in the same experimental room, participating in different foraging experiments. During Experiment 2 the sugar production by each feeder was always 69.48 mg h^{-1}, however the water component of the nectar was variable. This resulted in a trade-off between nectar concentration and nectar production rate. Feeders producing large volumes of nectar were also the most dilute and vice versa. The nectar concentrations could vary from 10 to 50% and were pseudo-randomly assigned to the feeders before the foraging sessions. During each 12-hour activity period, the nectar concentration assigned to each feeder remained constant. The mean nectar concentrations of the whole 24-feeder array varied between days (mean ± SD: 24.5 ± 4.1%, N = 16 days). As opposed to Experiment 1, in which reward delivery depended primarily on the state of the nectar account of a flower, in Experiment 2 visits with duration under 200 ms were never rewarded.

Behavioral measures

The behavioral data collected during the experiments were the time-stamped events of known duration of individually identified bats visiting specific feeders delivering rewards of known volume and sugar concentration. From these data a number of behavioral parameters were calculated for each bat for every experimental day. I focused on those aspects of foraging behavior that could automatically and objectively be quantified from the visit events data collected. The terms given in parentheses are the shortened parameter names, by which the variables will henceforth be referred to.

Spread evenness index (*spread index*) – a measure of how well the visits made by a bat were spread over the whole arrangement of feeders. It is based on Simpson's Equitability (ED) (Simpson 1949) calculated for each bat from its nightly visit events: $E_D = \frac{1}{\sum_i^S \left(\frac{n_i}{N}\right)^2} \cdot \frac{1}{S}$, where S = 24 is the total number of feeders, n_i is the number of visits the bat made at feeder i, and N is the total number of visits the bat made during the night. A value of 1 indicates that a bat visited all feeders equally, while visiting only a single feeder would lead to a value converging on 0.

Exploitation evenness index (*exploitation evenness*) – a measure of the evenness with which a bat exploited each of the feeders contained within its individual set of visited feeders. It is calculated as Simpson's Equitability (ED), but it differs from the previously mentioned spread index in that it is standardized by division by the total number of feeders actually visited by a particular bat instead of the total number of feeders available (S = 24). Calculated this way, it becomes a measure of individual preference between the visited feeders. Large values indicate indifference within the set, whereas small values indicate a preference for a few feeders only. For a bat visiting all 24 feeders, the parameters spread index and exploitation evenness were identical.

Mean spatial overlap (*Pianka's index*) – a measure of the degree of spatial overlap between the different individuals. To obtain Pianka's symmetrical index of niche overlap (Pianka 1973) the overlap for each bat with each of the other five bats was calculated and then a mean value was determined from these data for each individual. For example, Pianka's index between bats 1 and 2 was calculated as $\frac{\sum_i^{24} p_{i1} p_{i2}}{\sqrt{\sum_i^{24} p_{i1}^2 \sum_i^{24} p_{i2}^2}}$, where p_{i1} and p_{i2} are the relative visitation rates to the ith feeder made by bats 1 and 2, respectively. The maximum value of the index is 1 and indicates complete overlap, while the minimum value 0 indicates no overlap.

Preferred feeder constancy (*constancy*) – a measure of the preference of a bat for feeders it had visited during the previous day's foraging session. Calculated as –ln(1 – r), where r is the Pearson's correlation between the distribution of visits over the feeder array for one bat on the current day and on the previous day.

Visit duration (*duration*) – the mean of all logarithmically transformed visit durations made by a bat to feeders that delivered the full reward volume of about 54 µL. We restricted analysis to instances with this fixed reward volume because the two variables "reward

volume" and "visit duration" were positively correlated and individuals differed in their mean size of reward obtained. Longer durations of several seconds indicate a tendency to make clinging visits instead of the brief hovering visits, which normally lasted less than a second. However, there is no clear threshold duration value that separates the two behaviors.

Number of visits (*visits*) – the total number of feeder visits made by an individual bat.

Mean revisit time interval (*revisit time*) – the mean of all logarithmically transformed time intervals between the end of a bat's visit to a feeder and the beginning of the next visit to the same feeder. Feeders that were never revisited were discounted.

Revisit time interval variation (*cv revisit time*) – the coefficient of variation (CV) of the parameter revisit time for the different feeders visited by an individual.

Mean return cycle length (*revisit cycle*) – the mean number of other feeders visited before returning to the same feeder. Feeders that were never revisited or immediate revisits to the same feeder were discounted.

Spatial repeatability index (*spatial repeatability*) – the coefficient of variation (CV) of the parameter revisit cycle, multiplied by minus one. This parameter was suggested under the name "repeatability" by Thomson and colleagues (1997) as the most practical index to quantify the potential presence of habitual foraging routines, termed traplining by him. In order to avoid confusion with behavioral repeatability, I refer to this parameter as spatial repeatability. In the context of this experiment, this is a measure for the tendency of a forager to repeat the same visit cycles. More negative values indicate a lower spatial repeatability, and vice versa.

Number of revisits (*revisits*) – the absolute number of visits that were revisits, i.e. returns to the same feeder previously visited before visiting any other feeder.

Number of premature visits (*premature visits*) – the absolute number of visits to already emptied feeders. The amount of food available at a feeder was determined by its secretion rate and the time interval that had elapsed since a feeder was last visited and emptied by any of the six bats.

Reward variation (*cv reward*) – the coefficient of variation of the energetic contents of the rewards a bat received.

Foraging efficiency (*efficiency*) – the mean amount of kilojoules obtained per visit.

Time of first visit (*first visit*) – the logarithmically transformed time interval from the onset of the scotophase to the first visit a bat made at any feeder of the feeder array.

Number of feeders visited (*feeders visited*) – the total number of feeders visited by a bat.

Data Analysis

I was primarily interested in finding traits in which individuals showed significant consistency between the two experiments. Since the change in experimental conditions led to a significant change in some of the behavioral measures (see "Results"), I converted the row scores to standard scores to be used in further analyses.

I calculated the repeatabilities of individual behavioral measures, using the formula $r = S_A^2 / \left(S^2 + S_A^2\right)$, where S_A^2 is the variance among individuals and S^2 is the variance within individuals over time (Lessells and Boag 1987). I then used discriminant function analysis with bat individual as the grouping variable in order to identify the measures that were most important in distinguishing between the individual bats. I analyzed the list of behavioral measures using the forward stepwise method function greedy.wilks with the niveau parameter set to 0.05 from the klaR library in R 15.02 (R Development Core Team 2012). The order of entry in the model was taken as an indicator of the relative importance of the measures. For cross validation, I also performed a linear discriminant function analysis on the data from Experiment 1, with bat individual as the grouping variable. Behavioral measures were included in the model if they had high repeatabilities (>0.5) and if they were among the first five parameters to enter the stepwise analysis described above. As a minimum visit duration threshold of 200 ms was introduced before a reward was delivered in Experiment 2, bats with very short durations in Experiment 1shifted to higher durations, while bats making longer visits did not shift (data not shown). In order to correct for potential misclassification based on this parameter, I excluded it from classification analysis. I used the lda function from the MASS library in R (R Development Core Team 2012). I then used the obtained classification functions to classify the data points from Experiment 2 and calculated the hit ratio.

Table A1. Behavioral measures

Measures		Experiment 1 (N = 90)			Experiment 2 (N = 90)[a]		
	Repeatability	Mean	Median	SD	Mean	Median	SD
spread index	0.71	0.43**	0.43	0.13	0.39**	0.39	0.12
exploitation evenness[b]	0.52	0.61***	0.63	0.14	0.51***	0.50	0.10
Pianka's index	0.74	0.34	0.36	0.11	0.32	0.31	0.10
constancy	0.20	0.73***	0.75	0.21	0.84***	0.89	0.16
duration (ms)	0.76	984	1099	584	1066	1021	212
visits (N)	0.75	779	775	262	772	735	245
revisit time (s)	0.76	719	586	366	690	621	247
cv revisit time	0.38	1.68	1.57	0.60	1.76	1.75	0.32
revisit cycle (N of feeders)	0.86	13.52	13.35	3.85	13.38	13.92	4.69
spatial repeatability	0.37	–1.88**	–1.58	0.84	–2.10**	–2.04	0.59
revisits (N)	0.64	79.89***	46.00	71.91	32.12***	27.50	19.22
premature visits (N)	0.57	185***	144	121	105***	92	73
cv reward	0.51	0.88***	0.84	0.18	1.05***	1.04	0.14
efficiency (kJ visit^{-1})	0.37	0.068	0.070	0.016	0.070	0.067	0.020
time first visit (s)	0.38	827	190	1724	509	270	1376
feeders visited (N)	0.82	17.31	18	4.2	18.66	20	6.6

CV = coefficient of variation, SD = standard deviation, ms = milliseconds, N = absolute number, s = seconds, kJ = kilojoules. See "Methods" for calculations of unitless measures and general definitions. Differences marked with asterisks are statistically significant between the two experiments (Wilcoxon matched pairs test).

[a] In order to compare groups of equal size, the last experimental day was omitted from experiment 2 data in this analysis only.

[b] Underlined measures were the first five measures to be selected in a forward stepwise discriminant function analysis with bat individual as the grouping variable on the pooled data from both experiments.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Results

Bats maintained high flight activity in both experiments and made on average about 800 visits per individual and night (Table A1). The distribution of visits over the different feeders, as measured by the parameters *spread index*, *Pianka's index*, and *revisit cycle* was similar in the two experiments (Table A1). Except for *constancy*, *cv revisit time*, *spatial repeatability*, *efficiency*, and *time first visit*, all behavioral measures had repeatabilities of 0.5 or higher (Table A1). The order of entry of the behavioral measures in the forward stepwise discriminant function analysis (Wilk's Lambda = 0.00145, approx. F (45,772) = 31.06, $p <$ 0.001) was: *revisit cycle*, *duration*, *exploitation evenness*, *visits*, *Pianka's index*, *premature visits*, *revisit time*, *constancy*, *cv reward*, *time first visit*, *revisits*, *spatial repeatability*, *feeders visited*, *efficiency*, and *spread index*.

The discriminant function trained on the behavioral measures *revisit cycle*, *exploitation evenness*, *visits*, and *Pianka's index* from Experiment 1 (Wilk's Lambda = 0.01030, approx. F (45,772) = 87.40, $p < 0.001$) yielded a hit ratio of 97% when applied to the original data set. When the same function was used to classify the data points from Experiment 2, the hit ratio remained significant at 69% (Table A2). When all of the first five parameters to enter the stepwise model, including *duration*, were taken as inputs for the classification analysis, the hit ratios were 97% and 60% ($p < 0.001$), for Experiment 1 and Experiment 2, respectively.

Table A2. Discriminant analysis classification accuracy

Original Count	Predicted individual							
	Bat 1	Bat 2	Bat 3	Bat 4	Bat 5	Bat 6	% correctly classified[a]	Sex
Bat 1	3	12	1	0	0	0	18.75	female
Bat 2	0	13	3	0	0	0	81.25	male
Bat 3	11	0	5	0	0	0	31.25	female
Bat 4	0	0	0	16	0	0	100.00	male
Bat 5	1	2	0	0	13	0	81.25	female
Bat 6	0	0	0	0	0	16	100.00	male
Total	15	27	9	16	13	16	68.75[b]	

The discriminant functions were calculated from Experiment 1 data. Classification accuracy (hit ratio) was measured by using the same functions to classify data from Experiment 2.

[a] Individual and overall chance levels of correct classification are 16.67%

[b] $p < 0.001$, Randomization Test (Sokal and Rohlf 1995)

Discussion

Between the two experiments, bats showed high repeatabilities for most of the behavioral measures (Table A1). The individual behavioral consistency was observed despite the three-month time interval separating the two experiments. In this exploratory study, I propose that the behavioral measures that are most likely to reflect differences in behavioral types are *revisit cycle*, *duration*, *visits*, and *Pianka's index*. All of these measures had high repeatabilities (>0.7) and, with the exception of *duration*, the relative levels of the measures were good predictors of bat identity (Table A2). As explained in the "Methods", *duration* was rendered an unreliable predictor due to the introduction of a minimum visit duration threshold for reward delivery in Experiment 2. Bats 2, 3, and 6 responded to this change by increasing their *durations*, which distorted the relative differences among bats.

If we consider the correlational patterns among these parameters, the only significant correlations were between *visits* and *duration* and between *revisit cycle* and *Pianka's index* (Table A3). The negative correlation between *visits* and *duration* (r = –0.83, Table A3) indicates that bats making more visits also spent less time hovering in front of flowers (and were potentially also more likely to hover rather than make clinging visits). I suggest that *visits* and *duration* may reflect bats' *activity* behavioral syndrome (Réale et al. 2007). The positive correlation between *revisit cycle* and *Pianka's index* (r = 0.83, Table A3) indicates that the higher number of different feeders a bat visited before returning to a previously visited feeder, the more likely it was to have a large overlap with the preferred feeders of other bats. One possible interpretation is that both of these measures pertain to the *shyness-boldness* continuum (Réale et al. 2007), with bolder bats visiting more feeders and venturing more visits to flowers regularly visited by competitors. Both *revisit cycle* (r = 0.95, p = 0.003, N = 6) and *Pianka's index* (r = 0.85, p = 0.03, N = 6) correlate positively and strongly with

Table A3. Pearson correlation coefficients with uncorrected p-values in parentheses for standardized behavioral measures of the 6 bats. Correlations are based on average measures from all experimental days from both Experiment 1 and Experiment 2.

	revisit cycle	*duration*	*visits*	*Pianka's index*
revisit cycle	1			
duration	0.29 (0.58)	1		
visits	0.09 (0.87)	–0.83 (0.04)	1	
Pianka's index	0.83 (0.04)	0.58 (0.23)	–0.06 (0.91)	1

the measure *feeders visited*, suggesting that *feeders visited* could be another indicator of the same behavioral syndrome. Since the calculation of *feeders visited* is trivial in comparison to *revisit cycle* and *Pianka's index*, I suggest that it should be the preferred measure in studies with a higher number of animals or more complicated experimental procedures.

In a meta-study on the repeatability of behavior, Bell and colleagues report a higher repeatability in male vertebrates than females (Bell et al. 2009). It appears that, in consistence with these results and with the intuitive notion that females may considerably change their foraging behavior during pregnancy or lactation, male bats in this study had higher levels of repeatability than females (Table A2). However, the small sample size does not warrant a generalization of this finding. Furthermore, the sex differences in repeatability in the meta-analysis were primarily due to mate preference and only three of the studies in the analysis focused on foraging behavior (Bell et al. 2009). The dependence of repeatability on sex needs to be tested with a larger number of bats.

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CHAPTER 5: Cognition-mediated evolution: variance-sensitive behavior of a mammalian pollinator drives the evolution of lower nectar quality in virtual plants

Abstract. Plants pollinated by glossophagine bats produce unusually copious amounts of dilute nectars. This observation strongly contrasts with the preference of bats for sugar-rich nectars measured in both laboratory and field experiments. In light of this discrepancy the evolution of lower sugar concentrations in the nectars of glossophagine-pollinated plants from putative ancestors with higher nectar sugar concentrations remains a puzzle. Here I show that evolution towards lower food quality can be caused by a trade-off between food quality and food amount, if foragers evaluate reward options non-linearly. I presented computer-controlled artificial flowers with varying sugar concentrations and nectar production rates to groups of wild (*Glossophaga commissarisi*) and captive (*G. soricina*) nectar-feeding bats. In each experiment all flowers secreted the same amount of sugar over time but diluted it in different amounts of water. Bat choice determined the subsequent prevalence of the different flower types, with bats generally selecting for intermediate amounts of water. If nectar concentration (quality) evolves in response to nectar demand, increasing the number of flower visitors while retaining nectar productivity should result in stronger selection for higher amounts of water, as I demonstrate using groups of three and nine bats. Using computer-simulated bats I also show that the evolution towards lower nectar concentrations can be driven by bats employing non-linear reward evaluation methods. My results illustrate how the "price" of a commodity in a highly-dynamic system responds to increasing demand.

Introduction

There is an interesting discrepancy between the glossophagine bats' preference for sweeter nectars, with sugar concentrations as high as 50% weight/weight (see Chapters 1,3; Roces et al. 1993; Rodríguez-Peña 2007) and the more dilute nectars, 13-18% w/w, that are characteristic for plants primarily visited by glossophagines (Pyke and Waser 1981; von Helversen and Reyer 1984; Sazima et al. 1999; Perret et al. 2001; Tschapka 2004). This discrepancy becomes even more puzzling considering that many of the ancestors of phylogenetically distant glossphagine-pollinated plants must have had nectars with higher sugar concentrations (Fleming et al. 2009). Such evolutionary transitions from concentrated nectars to dilute nectars in direct opposition to the sugar concentration preferences of pollinators have also been described in bird-pollinated plants (e.g. Bolten and Feinsinger 1978; Pyke and Waser 1981; Tamm and Gass 1986; Stiles and Freeman 1993; Roberts 1996; Nicolson and Fleming 2003).

Despite intensive research and several proposed hypotheses (reviewed in Pyke and Waser 1981 and Nicolson and Fleming 2003), empirical evidence has not yet amounted to a coherent explanation for the origin and maintenance of lower nectar concentrations in vertebrate-pollinated plants. Researchers have suggested that (i) (suction-feeding) vertebrates achieve optimal energy intake rates at low viscosities (Baker 1975; Mitchell and Paton 1990; Kim et al. 2011); (ii) low concentrations deter nectar-robbing insects such as bees (Bolten and Feinsinger 1978); (iii) dilute nectars satisfy the water requirements of vertebrates (Baker 1975; Calder 1979); (iv) tubular flower shapes prevent water evaporation, maintaining nectars dilute (Plowright 1987); (v) in dilute nectars energy is partitioned in a way that results in more visits before the energy requirements of pollinators are satisfied (Martínez del Río et al. 2001; Ayala-Berdón et al. 2011); and (vi) dilute nectars are a consequence of increased expression of the enzyme invertase, which hydrolyses sucrose to glucose and fructose, increasing osmolality that draws in water from the nectary (Nicolson 1998, 2002; Nicolson and Thornburg 2007). Most likely a combination of factors has played a role in the evolution of low concentrations in vertebrate-pollinated plants; however empirical evidence either provides no support (i-iv) or is lacking for these hypotheses (v-vi). For instance, Pyke and Waser (1981) present data and analyses that are inconsistent with hypotheses (i-iii). Furthermore, unlike hummingbirds, bats extract flower nectar by repeatedly dipping and retracting their tongues in the viscous liquid, and the estimated optimal sugar concentration for the viscous dipping feeding mode is 52% w/w (Kim et al. 2011). Finally, many bat-

pollinated plants do not have tubular morphology but they still have low nectar concentrations (Sazima et al. 1999, Fleming et al. 2009) and within 45 different tubular-shaped species of Sinningieae (Gesneriaceae) the nectars of bat-pollinated flowers were found to be on average the most dilute (Perret et al. 2001).

Here I present experimental and simulation data collected to test the hypothesis that when plants invest equal amounts of sugar in nectar, but dilute it in different amounts of water, bat pollinators will select for lower nectar concentrations (and higher nectar secretion rates). This type of trade-off has been described for example in Sinningieae (Perret et al. 2001), in which plant tribe volume and dilution were found to increase by a similar factor so that total sugar production was comparable between flowers with different pollinators. Following Mitchell and Paton (1990) I refer to this trade-off in experimental settings as the "equal sugar presentation scheme". From an energetic point of view bats should be indifferent to differences in sugar concentration under the equal sugar presentation scheme. However, flowers that secrete dilute nectars reduce reward variance and may be perceived by bats as more rewarding because of diminishing returns in flower attractivity at higher sugar concentrations (Chapters 1–3). This effect can be explained with Jensen's inequality (Smallwood 1996; Shafir et al. 2003), which is often invoked to account for variance-sensitive behavior (Kacelnik and Bateson 1996; Kacelnik and Brito e Abreu 1998; Shafir et al. 2003) and states that the mean value of a function of a variable can differ from the value of the function evaluated at the mean variable. Thus, a bat using non-linear evaluation methods for both nectar concentration (Chapters 1,3) and nectar volume (Toelch and Winter 2007) may choose a flower with more dilute nectar rather than a typical insect-pollinated flower, because the increase in volume and probability to encounter a reward are more strongly evaluated than the decrease in concentration. If plants producing dilute but copious nectars receive more visits and have a greater pollination success, then low nectar concentrations would become more frequent over time.

The psychometric functions for concentration and volume discrimination in bats (Chapters 1,3; Toelch and Winter 2007) predict an aversion towards high reward variance, but reward variance itself depends on the foraging activity of the bats. When several bats compete for the same nectar sources, the variance each individual experiences is higher, because of the activities of other bats. The increased demand for higher nectar quantity could then cause a stronger selection against high reward variance and for lower nectar concentrations. From a biological market perspective (Noë and Hammerstein 1994, 1995), the bat density dependent

evolution towards lower nectar concentrations is predicted by the law of supply and demand, which states that the price of a commodity should increase if demand increases and supply stays constant. The "price" in this case is the number of visits a bat has to make per unit of sugar and it depends on the water component of a plant's nectar. If we assume that plants produce a constant amount of sugar in their nectar (representing the constant supply in the system) then the average price of nectar can be increased by diluting the nectar with water and decreased by making the nectar more concentrated. Although the price of nectar changes from the bats' perspective, the actual nectar production costs for the plants stay the same. Thus, the increase in demand (a higher number of foraging bats) should lead to a lower sugar concentration evolving in a population of flowers (higher equilibrium price of sugar). Alternatively formulated, the quality of nectar bats can "buy" for one visit should decrease in subsequent plant generations if there is an increase in demand.

Predictions about the direction and outcome of selection are difficult to test in natural plant populations, not only because of their long generation times, but also because of the lack of knowledge about genetic control of floral nectar traits (Mitchell 2004) and because of the difficulties associated with the experimental control over the studied traits. In order to avoid these setbacks the experimental approach employed in this project was *virtual pollination ecology*. The main concept of *virtual pollination ecology* is to expose an artificial population of the organism that is to be selected to real selection agents. The method stems from the *virtual ecology* concept, which was originally used to study the predator-prey interactions between blue jays (*Cyanocitta cristata*) and populations of digital cryptic moths (Bond and Kamil 1998, 2002). It has recently been employed in a study on the role of pollinator behavior in flowering plants ecological speciation (Gegear and Burns 2007) and in a study on the evolution of higher nectar secretion rates in bat-pollinated plants (Nachev 2007).

I conducted *virtual pollination ecology* laboratory and field experiments to examine how the foraging behavior of different groups of nectarivorous bats from the genus *Glossophaga* influence the evolution of nectar production rates and nectar concentration in a population of artificial flowers. I implemented the equal sugar presentation scheme, so that the artificial flowers had the same constant sugar production rate, but different water production rates. Each flower's water production rate was determined by a virtual genome, whose architecture was inspired by analyses of genetic control of nectar production rates in the monkey flowers *Mimulus lewisii* and *M. cardinalis* (Schemske and Bradshaw 1999). In all experiments I exposed ancestral populations of artificial flowers to live bats and used the

recorded virtual pollen transfer events as input to a genetic algorithm, which determined the frequency of the different water production rates in subsequent generations. Thus, the phenotypic trajectories of the coded trait could be tracked over several generations and compared to simulations of genetic drift. In order to test the prediction that an increase in nectar demand should lead to lower equilibrium sugar concentrations, I conducted laboratory experiments with groups of three and nine bats. Finally, in order to demonstrate the effect of non-linear reward evaluation on the evolution of nectar concentration, I used groups of three and nine simulated bats with linear and non-linear reward evaluation mechanisms and performed virtual pollination experiments similar to the ones conducted with real bats.

Methods

Laboratory experiments

Subjects

The experiments were conducted from April 2011 until March 2012 with adult Pallas's long–tongued bats (*Glossophaga soricina*) inμdividuals, bred in captivity within the same colony at Bielefeld University. Tests were made with two different groups of three individuals and one group of nine individuals. All bats were female, except for one male in the group of nine bats. They were marked with unique radiofrequency identification (RFID) tags using silicon necklaces and heat-shrinking tubing.

During every experimental night, bats received ad libitum water (to prevent dehydration in case of power failure), a total of three teaspoons of pollen, and a mixture of 1.2 g nectar concentrate for hummers (Nekton, Nektar Plus) and 1.8 g human infant follow-on formula (Alete Folgemilch 2) diluted in 6 ml of water. These supplementary nutrients were left in the room 60-30 min before the onset of the scotophase and were replaced on a daily basis before the next foraging session. The main source of food for the bats was nectar from the artificial flowers.

Artificial flowers and procedures

The flowers used in this experiment are thoroughly described elsewhere (Winter and Stich 2005). The experimental field consisted of 24 artificial flowers (4 rows × 6 columns) mounted pointing downwards on a horizontal rectangular frame suspended some 180 cm

above and parallel to the floor. The distance between flower "corollas" was 40 cm within the same row and between rows. The whole frame was positioned centrally inside the experimental room (535 × 468 cm). In addition to the 24 experimental flowers, two ad libitum 10% sugar concentration flowers were suspended on a tripod at the same height as the rest of the flowers, about one meter away from a corner of experimental field. The function of these flowers was to provide a constant food supply for bats that may have received insufficient food from the main flower array. Therefore visits to these "external" flowers were not analyzed. The climatic conditions were 22 °C and approximately 60% humidity. Light conditions were LD 12:12 and all experiments were conducted during the scotophase.

For regulation of reward amount and concentration each flower was equipped with two solenoid pinch valves and was connected via two separate tubing systems to two gas tight Hamilton glass syringes holding the nectar. Syringes were compressed automatically with stepping–motor–driven pumps. The smallest reward volume that could be pumped was about 1 µL. Nectar consisted of equal parts of sucrose, glucose and fructose dissolved in water as observed in natural nectars of glossophagine pollinated plants (Baker et al. 1998). The two pumping systems contained nectars with two different sugar concentrations, 10% and 50% w/w. The sugar concentrations delivered to each flower could be regulated by adjusting the ratio of the volumes of the two reward amounts delivered by each syringe pump. The mixing algorithm allowed for 21 different concentrations from 10 up to 50%, with a common difference of 2%. The accuracy of the mixing process had a SD of ± 3.5% units, estimated from experimentally delivered volumes using a hand–refractometer.

In order to mimic the variation in nectar volume bats experience in nature, I assigned virtual "nectar accounts" to every flower. The current state of the account determined the amount of nectar a bat could receive as a reward upon making a visit. During the nightly experiments the amount in this account increased continually at a constant production rate. The maximum reward a flower could deliver to a bat was 60 µl. If the account balance was smaller than the maximum value, the bat received the full amount remaining in the account. Feeders delivered no rewards until the minimum reward amount of 1 µL had accumulated. Thus, flowers could deliver different discrete reward volumes, ranging from 0 to the maximum reward (60 µL), with a common difference equal to the minimum reward (1 µL). The automatically recorded events consisted of the time at which a particular flower was visited, the identity of the bat, the duration of the visit, and the reward amount and sugar

concentration. Visits with duration under 200ms were never rewarded and were excluded from analysis.

Before each selection experiment bats were presented with a parental population of flowers under non-evolving conditions for three nights. These parental populations always had the same allele frequencies (within the same experimental condition) but the alleles were randomly combined in individuals and distributed on the feeder array. Throughout all experiments, bats were allowed to forage on the population of flowers for 12 hours during the scotophase. Nectar secretion was initiated two hours before the scotophase and continued throughout the foraging session, so that at the beginning of the session every flower had accumulated some nectar in its virtual account.

Genetic Algorithms

The virtual diploid genome of the flowers was set so that four diallelic genes simultaneously determined the nectar production rate and concentration. Sugar production rates were fixed and equal among flowers at 52.11 mg h^{-1} but water production rates were encoded by the genome, resulting in a trade-off between nectar energetic contents and nectar production rate. For the sake of simplicity I refer to phenotypes only by their sugar concentrations, not their corresponding nectar production rates. One gene (D) was responsible for 10% of the phenotypic variance and each of the other three genes was associated with 30% of the phenotypic variance (genes A through C). Genetic variation was additive and the individual contributions of the different alleles to the concentration were A = B = C = 7.5% units, a = b = c = 1.5% units, D = 2.5% units, and d = 0.5% units. This genome structure yielded a total of 21 different phenotypes (from 10% to 50% concentration with a common difference of 2% units) encoded by 81 different genotypes. The redundancy in the genome allowed for the maintenance of genetic variance in the population even after many rounds of selection. Reproduction entailed the transfer of virtual pollen among flowers. Every flower visit was interpreted as the removal of a single pollen grain from that flower. In meiosis the four genes assorted independently without crossing-over or mutation. Prior to pollen removal, a bat that already carried pollen would deposit its cargo for fertilization, generating a virtual seed, the product of a standard tetrahybrid cross between the parental pair of flowers. For the sake of simplicity the virtual plants were treated as self-incompatible and did not generate seeds when selfing. The different genotypes present in the pool of potential offspring generated by the visits of all bats during the 12-hour foraging session were ranked based on their frequencies. This ranking was then taken as the fitness measure for a Stochastic

Universal Sampling (SUS: Blickle and Thiele 1995; Bäck 1996) used to select the genotypes for the following generation. In order to minimize the effect of drift introduced by the SUS, SUS was repeated until the mean concentration of the candidate offspring population differed by no more than 0.3% units from the mean concentration of the complete pool of potential offspring. After this criterion was met, if necessary, allele fixation was prevented by replacing a single copy of the fixed allele in a random individual with the extinct allele, reintroducing it to the population. Finally, the new generated genotypes were randomly assigned positions on the experimental field.

For every group of bats I made two experimental runs, one with a high mean nectar concentration (36.67%, High run) and one with a low mean nectar concentration in the parental generation (14.17%, Low run). The duration of the runs was 50 generations in the group of nine bats, and between 15 and 22 generations in the groups of three bats. The cut-off criterion used in the groups of three bats is explained in the following section. The first group of three bats started the experiment with the High run and the second group of three bats and the group of nine bats started the experiment with the Low run first.

Analytical measures

The main dependent variables analyzed were the mean concentration and the response to selection in every generation. Response to selection was calculated as the mean concentration of the pool of potential offspring generated by bats minus the mean concentration of the population of flowers presented to the bats. For every experimental condition the mean concentration at equilibrium is the concentration for which the response to selection is zero. Selection towards higher concentrations is indicated by a positive response to selection and selection towards lower concentrations is indicated by a negative response to selection. The mean concentration at equilibrium was estimated by performing a linear regression with response to selection as the dependent variable and mean concentration as the independent variable.

The response to selection was also used to determine the cut-off criterion for the selection runs with three bats. A selection run was completed when it consisted of at least 15 generations and after the average response to selection over the past six days switched signs from positive to negative in the Low runs and negative to positive in the High runs. (This criterion was implemented for all runs after the first run (High) in the first group of three bats.)

In order to compare the observed phenotypic trajectories to the expected evolution in the absence of selection, I conducted a randomization selection test by shuffling the genotypes in the parental generation. I then took the real visitation and fertilization sequences in order to produce the following generation, using the same selection algorithm as in the actual selection experiment. The resulting offspring population was assigned random field positions and the visitation sequences from the following day were taken to generate the next step in this genetic drift simulation, until the desired number of generations was produced.

Field experiments

Field experiments were conducted from October to December 2007 with adult *Glossophaga commissarisi* Gardner bats on an experimental plot at La Selva Biological Station, Province Heredia, Costa Rica. Individual *G. commissarisi* (N = 16, nine males and seven females) were caught by mist-netting in the vicinity of feeders equipped with dimethyl disulfide odor decoys. After RFID-tagging bats were released on the site of capture, so that they were free to forage at the flower set-up or elsewhere in the rainforest. Non-marked bats were also attracted to the flowers, but since the identity and number of such visitors could not be determined, their visits were included in selection algorithm calculations as if they were made by a single individual. Nectar reward delivery was activated daily from 17:30h until 05:30h. Virtual nectar secretion was initiated one hour earlier, so that at the beginning of the experiments flowers had accumulated some nectar in their accounts.

The flower field used was the same as described in Chapter I, except that only 23 flowers were used. The overall procedure and methodology was the same as in the laboratory, unless otherwise specified. The smallest reward volume that could be delivered was about 4.5 μL and the maximum reward volume was 54 μL. Nectar consisted of one part sucrose and two parts fructose diluted in water. The sugar production rate of all flowers was 41.69 mg h^{-1}. SUS was not repeated until the mean concentration of the candidate offspring population differed by no more than 0.3% units from the mean concentration of the complete pool of potential offspring. Instead, I took the first candidate offspring population generated by SUS. The mean concentrations were 42.17% and 17.83%, for the High and Low runs, respectively. The High run was conducted first and lasted for 10 days and the Low run lasted for 14 days.

Simulations

Simulated environment

The environment consisted of 24 virtual flowers with properties similar to those of the artificial flowers described above. Flowers were ordered in a rectangular two-dimensional array, but bat choice was not spatially explicit (see below). The sugar production rate of all flowers was 26.055 mg h^{-1}. Nectar secretion and pollen production and dispersal occurred according to the previously described rules. The nectar secretion rates of the different sugar concentrations are shown in Fig. A1. Reward volumes were real values in the range of 0-60 microliters, 60 being the maximum amount a virtual bat could imbibe in one visit. Nectar secretion was initiated at time 0 and a virtual foraging session lasted for 12 hours. The selection algorithm was the same, except for a single modification introduced to prevent endless loops. In rare cases the criterion that the difference between the mean concentration of the candidate offspring population and that of the complete pool of potential offspring should be no larger than 0.3% units was found to be impossible to meet. Therefore, if no suitable offspring population was found after 1000 attempts, the difference threshold was increased by 0.1% units and SUS was repeated until the new criterion was satisfied.

Simulated bats

The time intervals (measured in seconds) between two flower visits were drawn from two lognormal distributions, approximating the time intervals observed in the groups of three bats in the laboratory. With probability (1 - ε) a sample was drawn from a distribution generating shorter intervals (μ = 3.2, σ = 1.8), otherwise the sample was drawn from a distribution generating longer intervals (μ = 5, σ = 2). The range of time intervals was restricted from 1 to 8000 seconds and the value of ε was set at 0.3. When a choice was being generated, the time interval was drawn first and then memory was consulted to select the next flower to fly to. The time distribution resulted in bats making (mean ± SD) 175 ± 36 visits per 12 hours. Bats could fly from any flower to any other flower in one second and inter-flower distance did not affect bat decisions.

The memory of a bat consisted of a vector of remembered values [V_{R1}, V_{R2}, ..., V_{Rn}], real numbers between 0 and 1, one for each flower. Remembered values were initialized at 0, so that the first visit was random.

In order to demonstrate the effect of non-linear reward evaluation on choice behavior, I investigated two different reward evaluation methods, while keeping all other procedures

unchanged. In the **linear evaluation** method, the reward obtained at a flower *i* was evaluated as follows:

$$V_{Ii} = 1.7857 \times (15.05 \times 10^{-6} v \times (0.05298c^2 + 9.56955c + 3.32727)), \quad (1)$$

where V_{Ii} is the instantaneous value, c is the concentration of the reward, the quadratic term converts concentration into grams of sugar per liter of solution (regression based on data from Haynes (2012)), v is the volume of the reward in microliters, 15.05 KJ/g is the energy density of the sugar mixture used in real bat experiments, and 1.7857 is a scaling constant. Thus, reward evaluation was linear with respect to the energetic content of a reward.

In the **non-linear reward evaluation** method instantaneous value was assumed to be the product of independent evaluations of volume and concentration. As discussed in Chapters 1–3, in accordance with (the near-miss to) Weber's law, the subjective perception of nectar volumes and concentrations can be modeled as an increasing but decelerating function (see also Shafir et al. 2003):

$$V_{Ii} = (1 - e^{-0.06v}) \times (1 - e^{-0.06c}), \quad (2)$$

where V_{Ii} is the instantaneous value, v is the volume of the reward, and c is the concentration of the reward. All subsequent steps were performed identically in both methods.

The remembered value V_{Ri} at time t_n for a feeder *i* was calculated as the average of the instantaneous values experienced at that feeder from the time of the first visit t_0:

$$V_{Ri}(t_n) = \frac{V_{Ii}(t_0) + \ldots + V_{Ii}(t_n)}{n(t_n)}, \quad (3)$$

where $n(t_n)$ is the total number of visits at feeder *i* up to t_n.

In order to avoid immediate revisits to flowers that have just been emptied, each remembered value was transformed to time-dependent remembered value (V^*_{Ri}) with the following revisit avoidance function:

$$V^*_{Ri} = (1 - e^{-0.02\Delta t}) \times V_{Ri}, \quad (4)$$

where Δt is the time elapsed since the last visit at that flower. Finally, the next feeder to be visited was chosen with Softmax action selection (Daw et al. 2006) as follows:

$$P_i = \frac{e^{V^*_{Ri}/\tau}}{\sum_{k=1}^{n} e^{V^*_{Rk}/\tau}}, \quad (5)$$

where P_i is the probability to select flower i, $n = 24$ is the total number of flowers, and τ is the parameter that determines how strongly choice relies on the vector of remembered utilities. In all simulations the value of τ was set at 0.15, in order to allow for sufficient exploration behavior, so that in groups of three, bats visited 17 ± 3 (mean ± SD) flowers, and in groups of nine they visited 23 ± 1 flowers.

Results

Laboratory experiments

In the first group of three bats individuals made 224 ± 70 (mean ± SD) visits at 13 ± 3 flowers per night during the High run and 251 ± 107 visits at 14 ± 4 flowers during the Low run. Similarly, in the second group of three bats individuals made 204 ± 40 visits at 16 ± 2 flowers per night during the High run and 257 ± 86 visits at 14 ± 3 flowers during the Low run. In the group of nine bats individuals made 697 ± 481 visits at 20 ± 6 flowers per night during the High run and 914 ± 592 visits at 19 ± 7 flowers during the Low run. In the first group of three bats 21% of all visits were revisits and did not produce potential offspring plants. In the second group of three bats the revisits were 17% and in the group of nine bats, only 5%.

In all Low runs the population of artificial flowers evolved towards higher nectar concentrations and the phenotypic trajectories were more consistent with directional selection rather than genetic drift (Fig. 1). In the High runs flowers evolved toward lower nectar concentrations and the phenotypic trajectories were statistically different from genetic drift in all groups, except in the second group of three bats (Fig. 1b). The responses to selection decreased with mean nectar concentration in the first group of three bats ($F(1,45) = 19.92$, $p < 0.001$, adj. $R^2 = 0.30$, Fig. 2a), in the second group of three bats ($F(1,37) = 19.36$, $p < 0.001$, adj. $R^2 = 0.33$, Fig. 2b), and in the group of nine bats ($F(1,100) = 55.71$, $p < 0.001$, adj. $R^2 = 0.35$, Fig. 2c). Within the same group of bats both runs converged towards an equilibrium concentration for which the response to selection was zero (Fig. 2, vertical dashed lines). The equilibrium concentrations were 28.9% in the first group of three bats, 32.5% in the second group of three bats, and 22.4% in the group of nine bats.

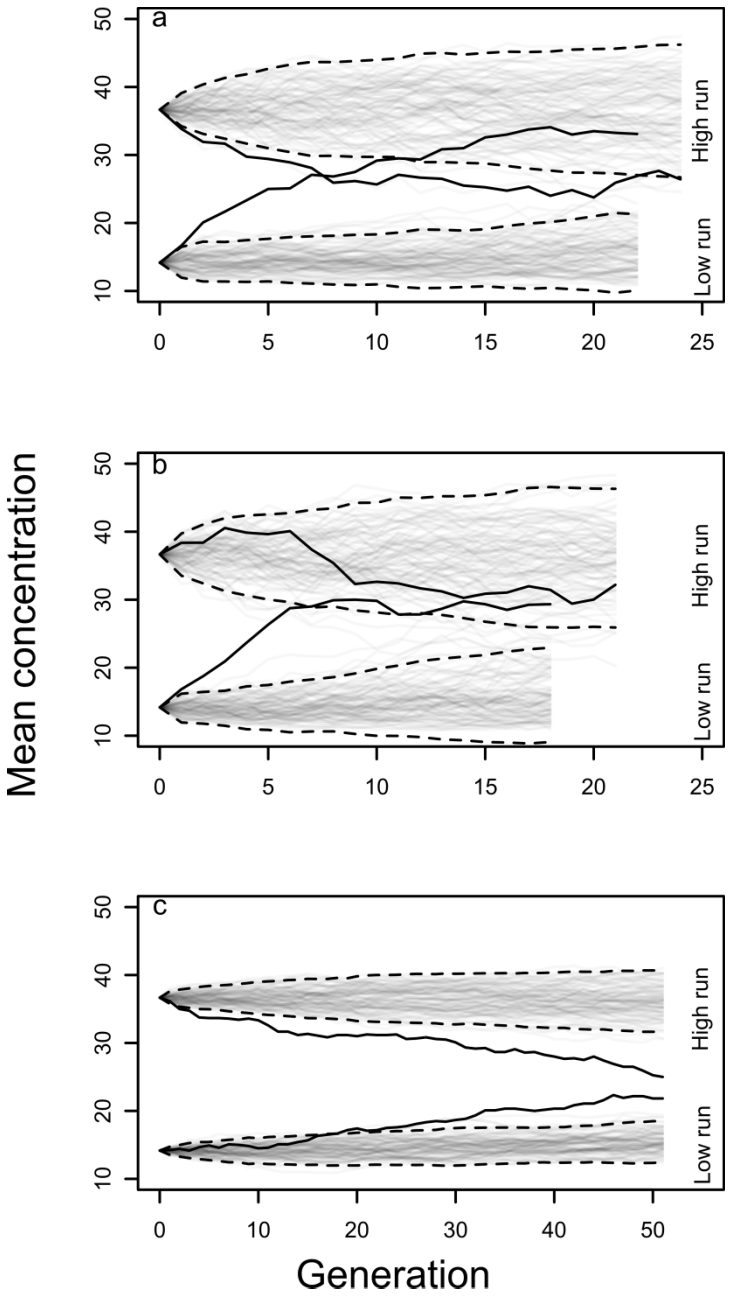

Figure 1. Phenotypic trajectories of populations of 24 artificial flowers evolving in response to visits by different groups of bats. The *abscissa* gives the generation number and the *continuous black lines* give the observed phenotypic trajectories in **a** the first group of three bats, **b** the second group of three bats, and **c** the group of nine bats. *Gray lines* are individual runs of genetic drift simulations and the *dashed black lines* give the 95% prediction intervals from 100 simulation runs. When an observed phenotypic trajectory lies outside of the prediction interval, the results are significantly different from evolution caused only by genetic drift

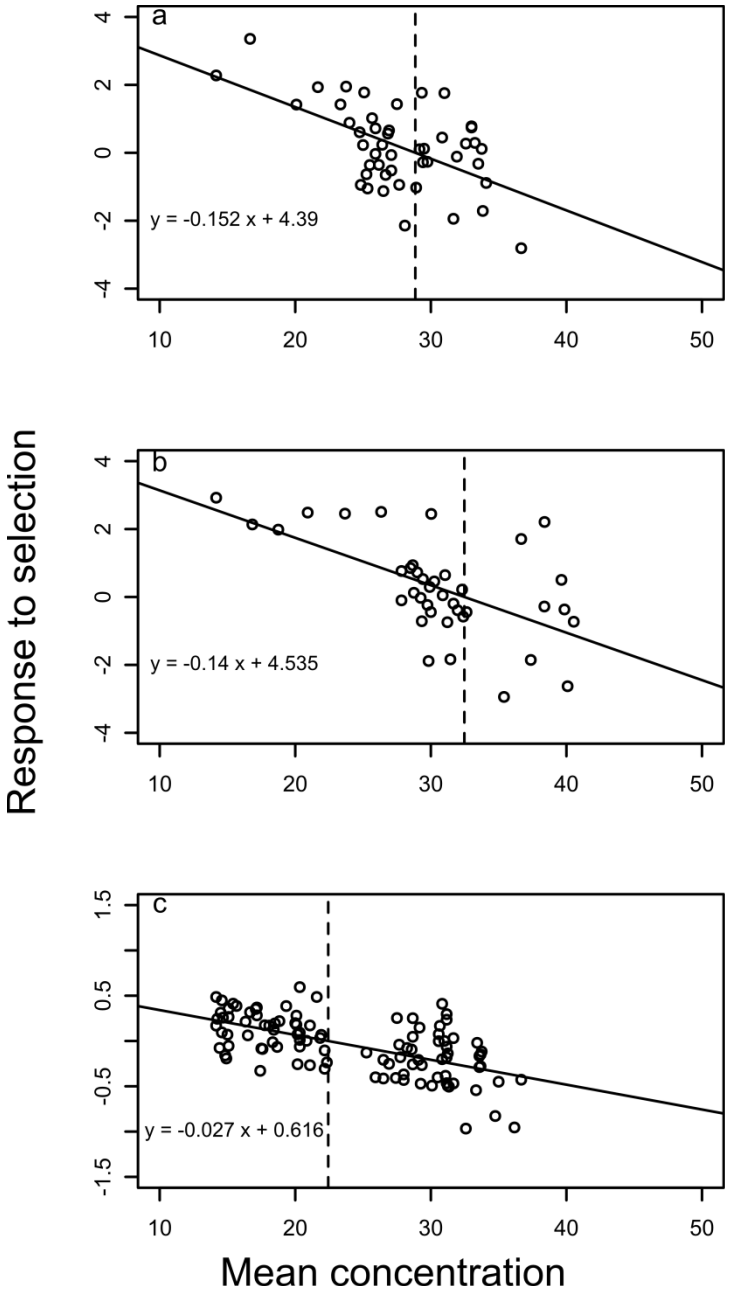

Figure 2. Responses to selection in populations of 24 artificial flowers evolving in response to visits by different groups of bats. The *abscissa* gives the mean sugar concentration in the population and the *symbols* give the response to selection, calculated as the difference between the mean concentration of the pool of potential offspring generated by bats and the mean concentration of the population of flowers presented to the bats. Data are shown for **a** the first group of three bats, **b** the second group of three bats, and **c** the group of nine bats. *Continuous lines* give the linear regression fits (see text for details). *Vertical dashed lines* give the intercepts with the abscissa, which correspond to the mean concentrations at equilibrium. The *linear regression equations* are given in the lower left corner

Field experiments

A total of 29845 flower visits (range: 464-2778 visits per night) were recorded over the time period of the selection trials (N = 24 days, excluding the training non-selection days). During the High run there were 1706 ± 520 visits per night (mean ± SD) and during the Low run there were 772 ± 326 visits per night. All flowers were visited every night, with tagged bats visiting on average 16 ± 7 flowers. The mean proportion of visits per night performed by tagged bats was 47% (range: 6-74%). On average, three tagged bats visited the flower field per night (range: 1-6), whereas seven of the 16 tagged bats were never detected after they were released. In general, individual bats that visited the flowers would either stay and forage throughout the night (1-3 animals per night) or only make a few visits and leave. Only 2.7% of all visits were revisits and did not produce potential offspring plants.

Bats caused the population of artificial flowers to evolve towards lower concentrations in the High run and towards higher concentrations in the Low run (Fig. 3). The responses to selection decreased significantly with mean nectar concentration ($F(1,23) = 23.18$, $p < 0.001$, adj. $R^2 = 0.48$, Fig. 3b). The two phenotypic trajectories converged to an equilibrium concentration of 35.7%.

Simulations

The pattern of evolution observed when real bats selected the nectar concentrations of the artificial flowers was reproduced using virtual bats with non-linear reward evaluation methods. In the Low simulated runs virtual bats selected for higher nectar concentrations and in the High runs they selected for lower concentrations (Fig. 4). Regardless of the mean concentration of the starting parental generation the population converged on the same equilibrium concentration within 100 generations. In the group of three bats the equilibrium concentration was 26.5% (Fig. 4; Fig. 5a), which was higher than 16.2%, the equilibrium concentration in the group of nine bats (Fig. 4; Fig. 5b).

When simulated bats used linear reward evaluation methods the pattern of evolution was different. In the group of three bats there was selection for higher concentrations in both the High and the Low runs, converging on 41.5% (Fig. 6; Fig. 7a). In the group of nine bats there was no selection in either of the two runs (Fig. 6; Fig. 7b).

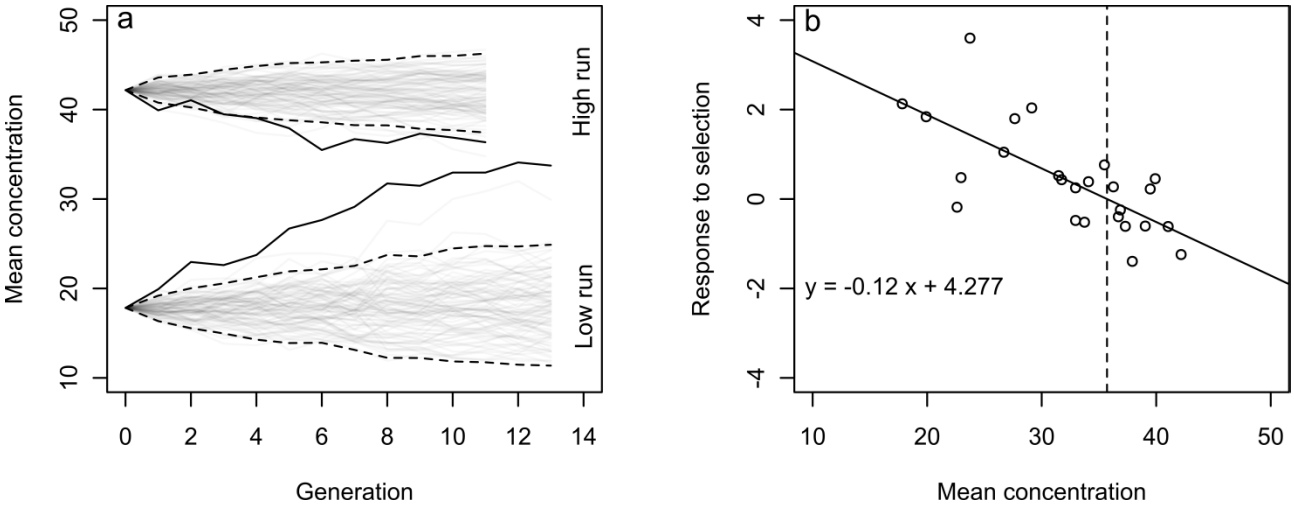

Figure 3. Virtual evolution of a population of 23 artificial flowers evolving in response to visits by free-flying wild bats. **a** Phenotypic trajectories. **b** Responses to selection. Same notation as in Fig. 1 and Fig. 2

Discussion

In their choice among different flower types captive and wild bats avoided flowers whose nectar was too dilute and flowers that secreted nectar too slowly, exerting selection for flowers with intermediate water production rates (Fig. 1; Fig. 3a). The equilibrium sugar concentrations after bat-exerted selection (Fig. 2; Fig. 3b) were lower than the concentrations associated with the highest sugar intake rates (Roces *et al.* 1993, Kim *et al.* 2011) attainable with the chosen virtual genome. Virtual evolution occurred in the absence of direct fitness costs of nectar production, as all artificial flowers had the same sugar production rate, and in the absence of nectar robbers.

This evidence provides support for the hypothesis that evolutionary transitions from high to lower sugar concentrations can be driven by the choice behavior of pollinators, even if pollinators prefer sweeter nectars over more dilute ones when presented with unlimited-volume choices. Thus, when considering the evolutionary origin of an unexpected trait, it is important to take into account its genetic architecture and potential pleiotropic effects. The trait under selection in this case was not the sugar production rate, but the water content of floral nectar. In natural plants lower sugar concentrations may have evolved as a byproduct of selection for higher nectar secretion rates. These results also provide indirect support for the hypothesis that in the transition from insect to bat pollination plants have been selected for increased expression of invertase (see “Introduction”). Even though the hexose to sucrose

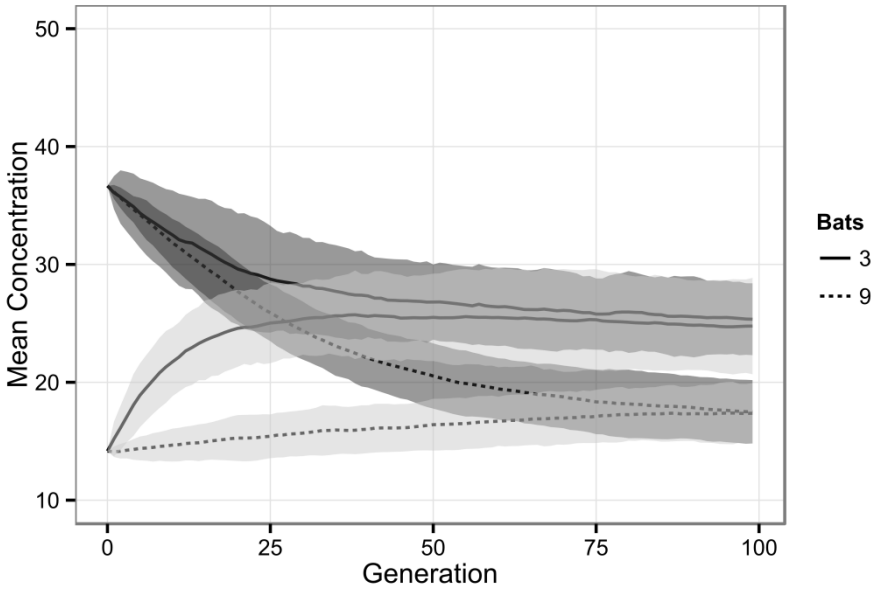

Figure 4. Phenotypic trajectories of populations of 24 virtual flowers evolving in response to visits by different groups of virtual bats with non-linear reward evaluation methods. The *abscissa* gives the generation number, *continuous lines* give the mean phenotypic trajectories of groups of three bats over 100 replicates, and *dashed lines* give the mean phenotypic trajectories of groups of nine bats. *Light gray ribbons* around lines give the 95% prediction interval for Low run simulations and *dark gray ribbons* give the 95% prediction intervals for High run simulations. Ribbons are transparent, resulting in different shades of gray in overlapping regions

ratio was kept constant throughout all experiments in this study, the intake responses of glossophagine bats suggest that they perceive different hexose and sucrose mixtures as energetically equivalent (Rodríguez-Peña et al. 2007; Ayala-Berdón et al. 2008; but see also Herrera 1999). The prediction is therefore that the same pattern of selection would be observed if the 50% nectar source is made from pure sucrose and the 10% source from a 1:1 glucose-fructose mixture.

In accordance to the laws of supply and demand (Noë and Hammerstein 1994, Noë and Hammerstein 1995), nectar sugar production rates (Figs A2–A5) and number of pollinators (Figs 4–7) appear to be important factors modulating the selection pressure for nectar water contents under the equal sugar presentation scheme. If nectar secretion rates are sufficiently high or the demand is low, then flower types differ primarily in concentration and selection favors higher concentrations and lower secretion rates. Conversely, if nectar secretion rates are low or the demand is high, slow-secreting flowers are much more likely to be empty and selection favors higher secretion rates and lower concentrations. When shifting

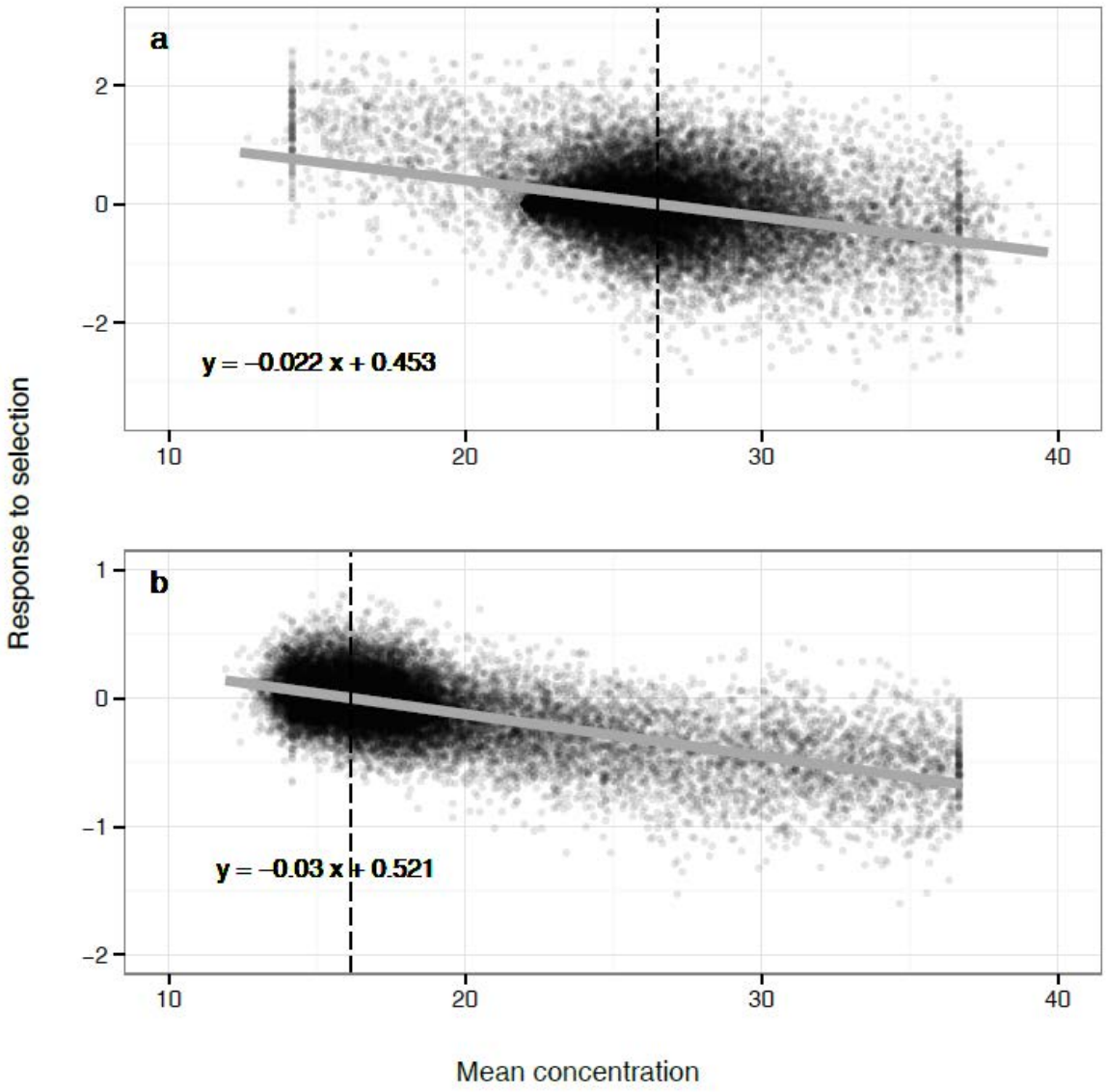

Figure 5. Responses to selection in populations of 24 virtual flowers evolving in response to visits by different groups of virtual bats with non-linear reward evaluation methods. The *abscissa* gives the mean sugar concentration in the population and the *symbols* give the response to selection, calculated as the difference between the mean concentration of the pool of potential offspring generated by bats and the mean concentration of the population of flowers presented to the bats. Data are shown for **a** groups of three bats, **b** groups of nine bats. *Continuous gray lines* give the linear regression fits (see text for details). *Vertical dashed lines* give the intercepts with the abscissa, which correspond to the mean concentrations at equilibrium. The *linear equations* are given in the lower left corner

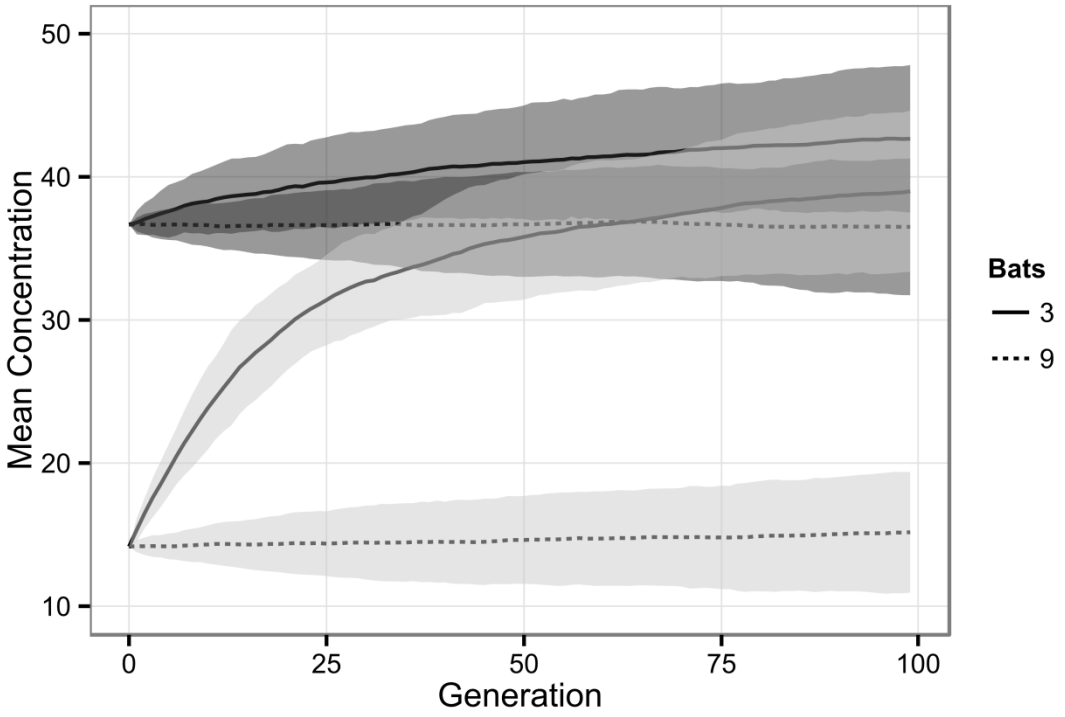

Figure 6. Phenotypic trajectories of populations of 24 virtual flowers evolving in response to visits by different groups of virtual bats with linear reward evaluation methods. Data are shown for **a** groups of three bats, **b** groups of nine bats. Same notation as Fig. 4

from smaller to larger pollinators, plants also experience an increase in nectar demand and are therefore expected to increase the price of their nectar, i.e. evolve lower nectar concentrations. Indeed, Pyke and Waser (1981) describe that nectar concentration tends to decrease as body size increases for different groups of both vertebrate and invertebrate pollinators. They suggest that the average size of pollinators plays a very important role with respect to both nectar volume and nectar concentration. However, it remains to be shown whether this trend holds within glossophagine bats. Data by Rodríguez-Peña et al. (2007) suggest that the larger and more specialized *Leptonycteris yerbabuenae* can discriminate differences in sugar concentration better than the smaller and more generalist *G. soricina*. It may be the case that specialization on nectar-feeding causes an increased selection pressure for better sugar concentration discrimination that can mask or even reverse the trend predicted by body size.

As the results from the simulations demonstrate (Figs 4–7), pollinators only select for lower nectar concentrations, if they are variance-sensitive, i.e. if they use non-linear reward evaluation mechanisms. As discussed in Chapters 1–3, the perceived attractiveness of flowers with high concentrations seems to increase with diminishing returns, so that an increase in nectar production rate can offset a decrease in sugar concentration. Therefore the degree to

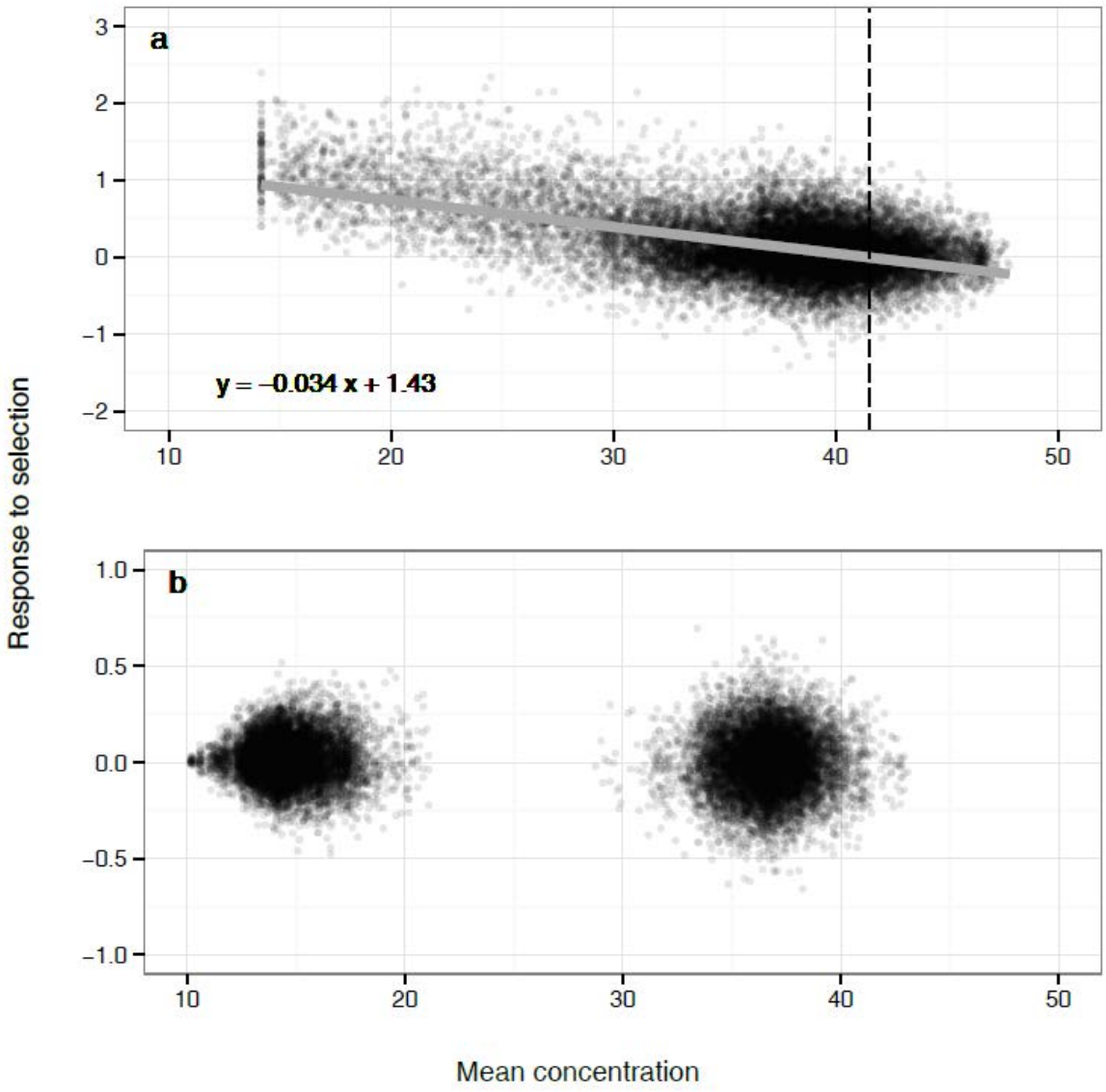

Figure 7. Responses to selection in populations of 24 virtual flowers evolving in response to visits by different groups of virtual bats with linear reward evaluation methods. Data are shown for **a** groups of three bats, **b** groups of nine bats. Same notation as Fig. 5

which pollinators differ in their variance sensitivity is also expected to modulate the selection pressure for or against low nectar concentrations.

With the chosen genome model for nectar water contents the additive effects of genes redundant in their large phenotypic effects maintained a relatively high level of genetic variance in the population in spite of repeated generations of selection (data not shown). In natural populations, such genetic variance can play a key role in allowing closely related species to easily shift from one pollination syndrome to another (Schemske and Bradshaw 1999; Gegear and Burns 2007; Thomson and Wilson 2008; Smith 2010; Martén-Rodríguez et

al. 2010) by responding to changes in nectar demand. In conclusion, the results of this study demonstrate a plausible mechanism for the evolutionary transition from high to low nectar sugar concentrations in bat-pollinated plants and by extension, in plants switching from a smaller (e.g. bees) to larger pollinators (e.g. birds).

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Chapter 5: Appendix

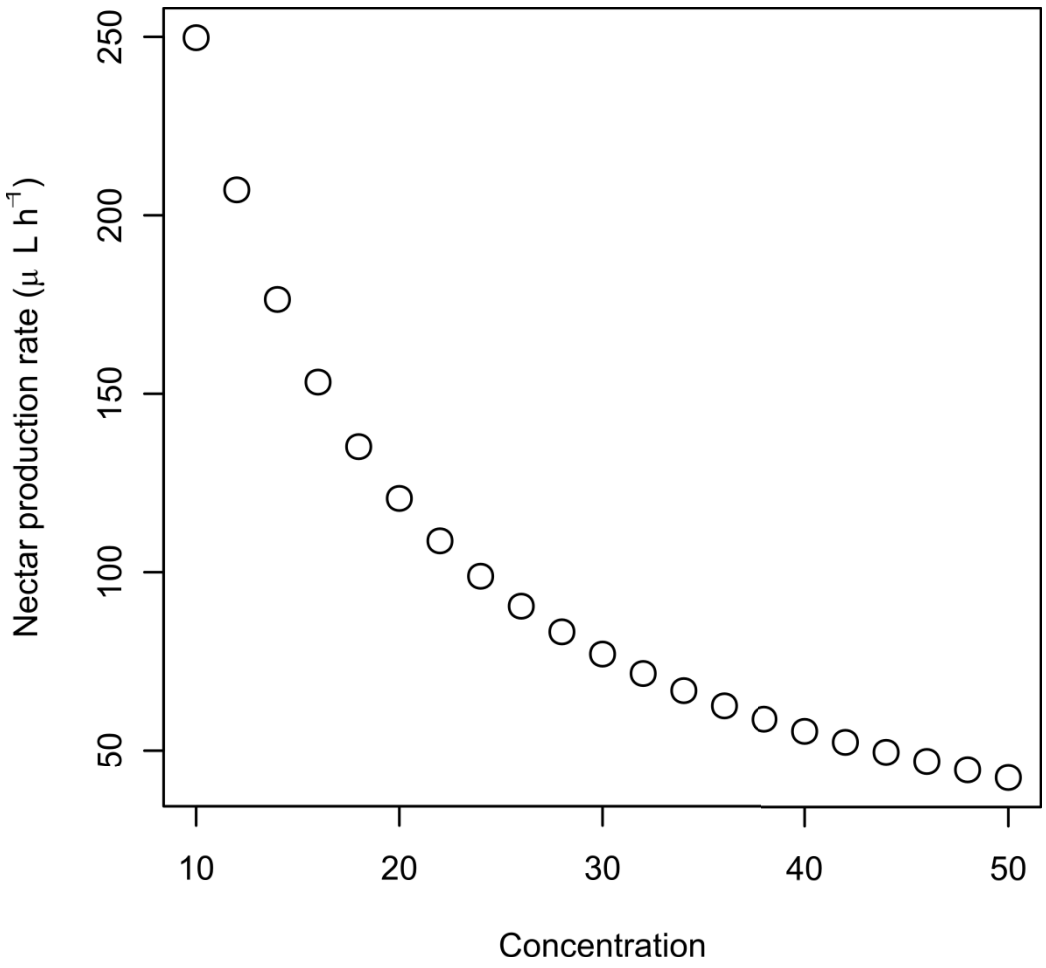

Figure A1. Trade-off between nectar sugar concentration and nectar production rate in the virtual flowers. All flowers have the same sugar production rate (26.055 mg h^{-1}), but fast-secreting flowers have lower concentrations and slow-secreting flowers have higher concentrations.

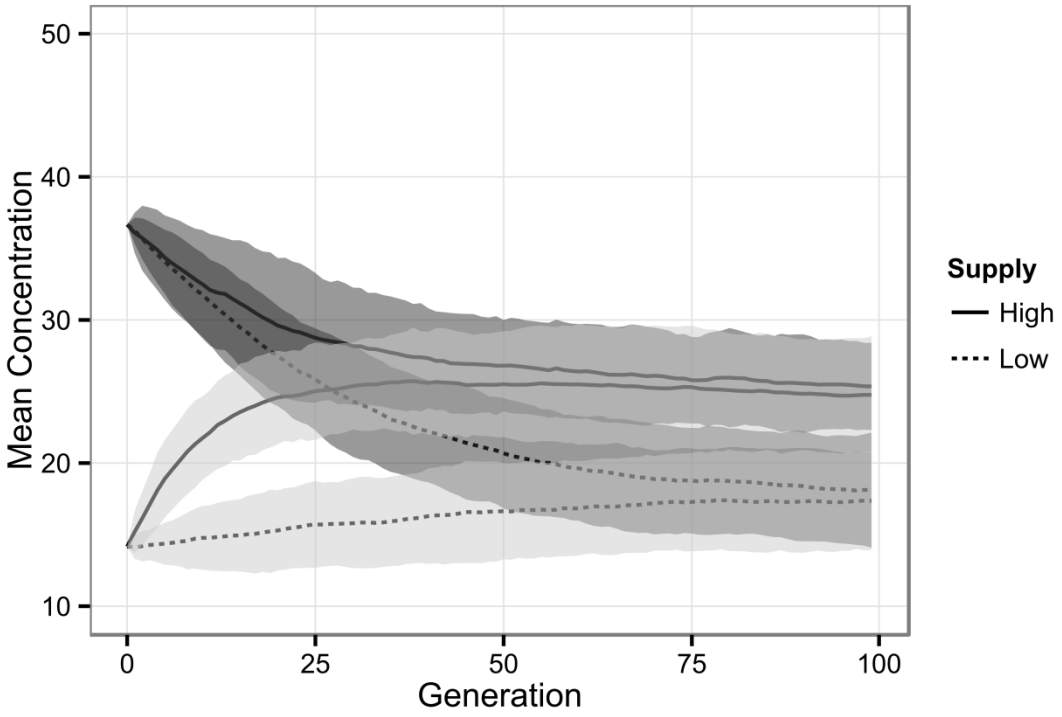

Figure A2. Phenotypic trajectories of populations of 24 virtual flowers with different sugar production rates evolving in response to visits by groups of 3 virtual bats with non-linear reward evaluation methods. Data are shown for **a** a population of flowers with High sugar production rates (26.055 mg h^{-1}), **b** a population of flowers with Low sugar production rates (8.685 mg h^{-1}). Same notation as Fig. 4

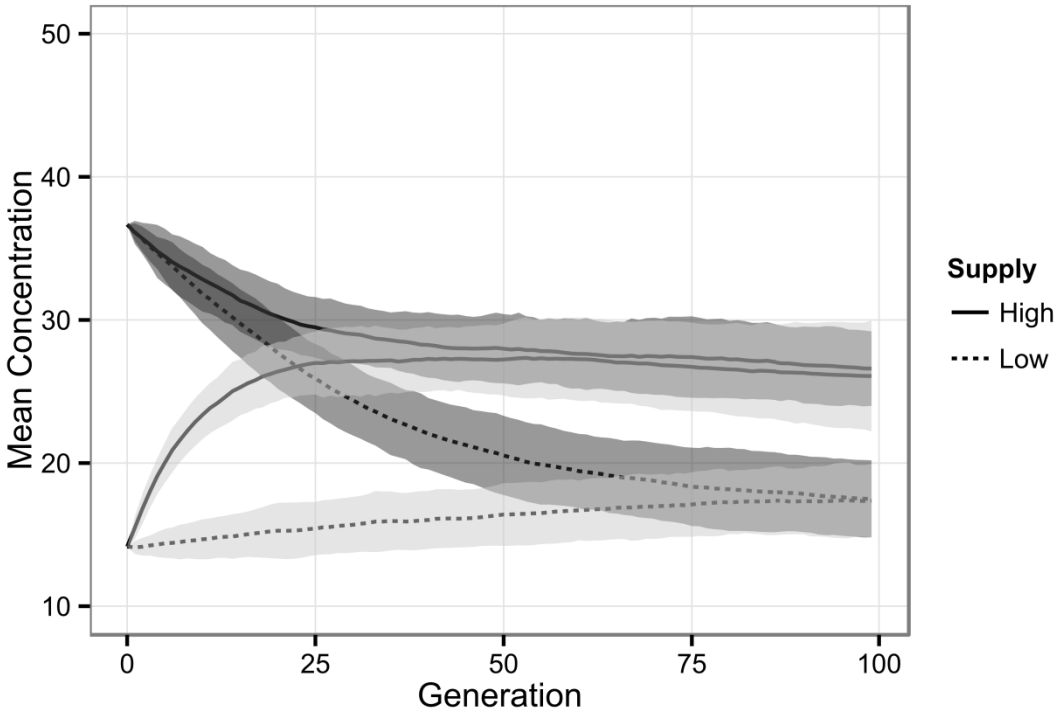

Figure A3. Phenotypic trajectories of populations of 24 virtual flowers with different sugar production rates evolving in response to visits by groups of 9 virtual bats with non-linear reward evaluation methods. Data are shown for **a** a population of flowers with High sugar production rates (78.165 mg h^{-1}), **b** a population of flowers with Low sugar production rates (26.055 mg h^{-1}). Same notation as Fig. 4

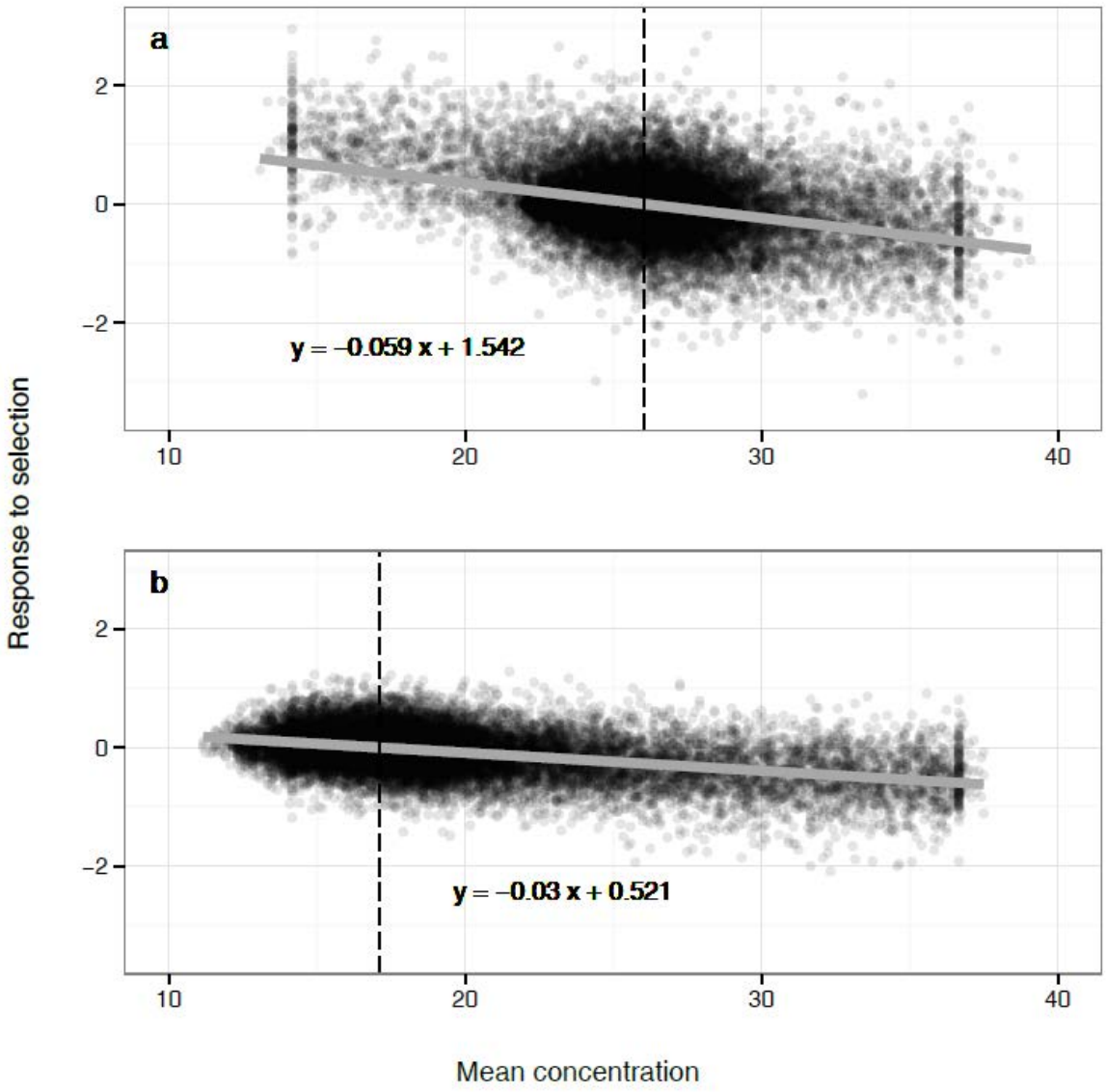

Figure A4. Responses to selection in populations of 24 virtual flowers with different sugar production rates evolving in response to visits by groups of 3 virtual bats with non-linear reward evaluation methods. Data are shown for **a** a population of flowers with High sugar production rates (26.055 mg h^{-1}), **b** a population of flowers with Low sugar production rates (8.685 mg h^{-1}). Same notation as Fig. 5

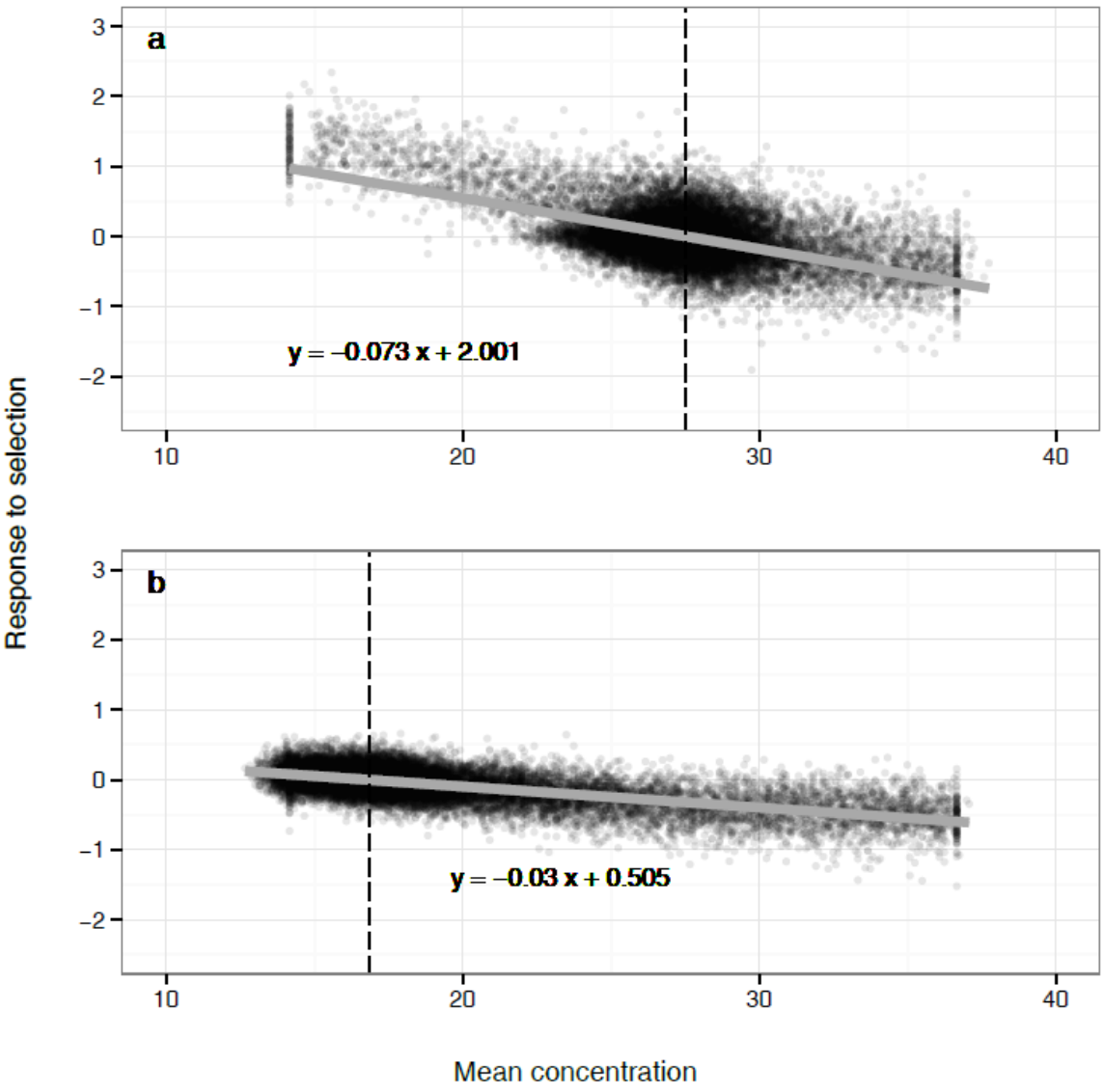

Figure A5. Responses to selection in populations of 24 virtual flowers with different sugar production rates evolving in response to visits by groups of 9 virtual bats with non-linear reward evaluation methods. Data are shown for **a** a population of flowers with High sugar production rates (78.165 mg h^{-1}), **b** a population of flowers with Low sugar production rates (26.055 mg h^{-1}). Same notation as Fig. 5

Conclusions and future directions

In this thesis I argue that the ecological and evolutionary interactions between plants and their animal pollinators can be best understood if we focus on the transfer of information from the physical, objective nectar reward properties to the cognitive architecture of the pollinator, through which choice is effected. This cognitive ecology of pollination approach (Chittka and Thomson 2001; Shafir et al. 2003) emphasizes the constraints that sensory and cognitive processes for encoding profitability pose on foraging behavior. As discussed in Chapters 1-3 the capacity of different nectar-feeding animals to discriminate among sugar concentrations seems to follow the same (non-linear) functional relationship with respect to the relative intensity of the presented stimuli. The fitted psychometric functions allow for parameters to be compared between different pollinator groups, revealing that animals that respond more strongly to smaller differences in sugar concentration also tend to pollinate flowers with sweeter nectars (Chapter 3). To the best of my knowledge, this is the first comparative study using this approach. I expect future data acquired from a wider range of taxonomic groups will provide even stronger evidence for pollinator-exerted selection on floral nectar concentration.

Here I also present the first direct experimental support for the adaptive function of low nectar concentrations in bat-pollinated plants. As discussed in Chapter 5, lower nectar selections seem to evolve as a result from the trade-off between sugar concentration and nectar production rate and the non-linear reward evaluation mechanisms employed by pollinators. At first glance, it appears that these results are contradicted by the analysis presented in Chapter 1, according to which, for equivalent changes in caloric value bats are predicted to be more sensitive to changes in concentration than to changes in volume. However, the analysis in Chapter 1 only considered the case in which choice options gave constant reward amounts, whereas reward amounts were dynamically varied in the experiments described in Chapter 5. It is therefore important to consider how the experience of encountering an empty flower affects reward expectations. At least in bumblebees, it appears that no updating of reward expectation occurs upon encountering an empty flower (Chapter 2). No such analysis has been performed with bats. If bats and other vertebrates do update their reward expectations when experiencing non-rewarded visits that would be

another factor that promotes the evolution of lower nectar concentrations in vertebrate-pollinated plants.

As pollinators become more specialized in feeding on nectar, their cognitive architecture dedicated to the evaluation of nectar is also expected to become more specialized. The dependence of discrimination performance on the magnitude of the presented stimuli (Weber's law or near miss to Weber's law) is a common observation in various sensory systems and across taxonomic groups (Deco and Rolls 2006; Kacelnik and Brito e Abreu 1998; Walsh 2003). Therefore, it most likely represents a phylogenetically conserved trait that already occupies an adaptive peak (Sinn 2003; Portugal and Svaiter 2010) even though it may not always generate optimal choices (Livnat and Pippenger 2008; Fawcett et al. 2012). If we assume that the near-miss to Weber's law is a good predictor of discrimination performance of sugar concentrations, past selection for better nectar foraging efficiency can be detected as the decrease in the threshold of the psychometric function and as the increase in its slope (Chapter 3). This hypothesis can be tested by comparing these parameters in pollinators that show different degrees of specialization on nectar feeding. On the other hand, the small magnitude effect in bees and the large magnitude effect in vertebrates are more likely to result from morphological or phylogenetic constraints than being a specifically selected trait from natural selection.

Different foraging strategies may have similar fitness payoffs but be better adapted to different seasonal and local habitat conditions, e.g. to different flower distributions, phenologies, and nectar production patterns. The individual differences in foraging behavior described in Chapter 4 and its Appendix suggest that in the foraging context the behavioral plasticity of *Glossophaga* is constrained, which raises the question of how behavioral variance is maintained in natural populations. A potentially fruitful avenue for future research is the use of agent-based models that incorporate the details of proximate cognitive mechanisms (Fawcett et al. 2012) such as the one described in Chapter 5. For instance, the psychometric functions estimated for different pollinators can be used as the basis for a comparative reward evaluation mechanism, in which the lapse rate is explicitly incorporated as the τ parameter from Eq. 5 in Chapter 5. The three psychometric function parameters, threshold, slope, and lapse rate, can then be encoded in a virtual genome and assigned to groups of pollinators competing against each other for the same sources of nectar. With the application of a suitable genetic algorithm, the optimal combinations of these parameters can be computed under different resource distributions and supply-demand ratios. This approach

also allows for virtual co-evolution experiments to be performed, in which plants and pollinators are both exerting selection on each other, "bargaining" for the optimal price of nectar.

In summary, the cognitive ecology of pollination approach together with the virtual pollination ecology methodology provide an opportunity to tests previously untestable or not easily testable hypotheses on neuroeconomics, foraging behavior, competition, and plant-pollinator interaction dynamics, especially in terms of the underlying mechanisms promoting gene flow between plants.

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List of contributions

Chapter 1: This study was supervised by and designed with York Winter. Field work assistance provided by Arne Jungwirth. Experimental software was programmed by Alexej Schatz.

Chapter 2: This study was designed with York Winter and performed in collaboration with James Thomson. Vanessa Rojas Lenguas provided assistance with the experiments. Experimental software was programmed by Alexej Schatz.

Chapter 3: This study was supervised by and designed with York Winter, and performed in collaboration with Kai Petra Stich. Experimental software was programmed by Alexej Schatz.

Chapter 4: This study was supervised by and designed with York Winter. Anika Beer and Johanna Cieslak provided assistance with the laboratory experiments. Experimental software was programmed by Alexej Schatz.

Chapter 5: This study was supervised by York Winter and performed in collaboration with Kai Petra Stich. The study was designed with the help of York Winter, Alan Bond, Alan Kamil, and Klaus Reinhold. Experimental software was programmed by Alexej Schatz. Core routines for the simulation software were developed by York and Clemens Winter.

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